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Effect of insulin-induced hypoglycemia on phosphofructokinase and pyruvate kinase in regions of rat brain

(Key words : insulin/hypoglycemia/brain/glycolysis)

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Abstract

The effect of insulin-induced hypoglycemia of varying duration on the activity of phosphofructokinase and pyruvate kinase in different regions of rat brain was studied. Pyruvate kinase showed significant decrease in activity in the three brain regions, viz., cerebral hemispheres, cerebellum and brain stem after one hour of insulin administration. Phosphofructokinase activity also decreased in cerebellum and brain-stem after insulin administration. The enzyme activities show a general trend of reversal to control values after three hours of insulin administration. The results suggest that glycolysis may be regulated in the brain at the level of phosphofructokinase and pyruvate kinase under limited substrate availability as is present in hypoglycemia.

Introduction

Insulin-induced hypoglycemia leads to profound central nervous system disturbances, and is accompanied by a decreased cerebral uptake and utilization of glucose[1, 2]. It reduces the brain content of free and bound glucose, glycogen, lactate and citrate[3]. A reduction in the flux of metabolites through the glycolytic pathway and TCA cycle in the brain of rat and mice has been reported under hypoglycemic conditions[4–7]. Disturbances in amino acid levels in brain have also been shown to occur following insulin-induced hypoglycemia[4, 8].

Gorell *et al.*[5] have reported that hypoglycemic stupor may be due to a specific lack of glucose or one of its metabolites. Hexokinase, phosphofructokinase and pyruvate kinase are key control points of glycolysis in brain[9, 10] and a redistribution of brain hexokinase isozyme

under hypoglycemia has been reported by Kaur *et al.*[11]. As rat brain phosphofructokinase and pyruvate kinase activities have been shown to be modulated by conditions of insulin deficiency such as in alloxan-induced diabetes,[12] it was thought of interest to observe how these enzymes adapt themselves to changing substrate availability obtained during insulin-reduced hypoglycemia. In the present communication, the changes in the activities of phosphofructokinase and pyruvate kinase were analysed in three brain regions, viz., cerebral hemispheres, cerebellum and brain stem during insulin-induced hypoglycemia of varying durations.

Materials and Methods

Adult female rats of Wistar strain weighing between 200-220 g were used. The experimental rats were injected intraperitoneally with 5 units/100 g body weight of insulin (Lente, Boots Co., India) in physiological saline. The animals were sacrificed by cervical dislocation after one, two and three hours of insulin administration. Cerebral hemispheres, cerebellum and brain stem were dissected and kept on ice. The tissues were homogenized in a Potter Elvehjem type homogenizer fitted with a Teflon plunger in 9 volumes 0.25 M sucrose containing 0.02 mM triethanolamine and 0.1 mM dithiothreitol (pH 7.4). The extracts were centrifuged at 12,000 xg for 40 minutes and the supernatants were taken for enzyme assays.

The activities of phosphofructokinase (ATP : D-fructose-6-phosphate 1-phosphotransferase. EC 2.7.1.11) and pyruvate kinase (ATP : pyruvate phosphotransferase, EC 2.7.1.40) were determined by measuring the disappearance of NADH in an assay system coupled with α –glycerophosphate dehydrogenase-triosephosphate isomerase and lactate dehydrogenase respectively, as described by Srivastava and Baquer[12]. One unit of the enzyme activity is defined as 1 μmole of NADH oxidized per minute per gram tissue at 25°C.

Blood glucose concentrations were measured enzymatically using hexokinase and glucose-6-phosphate dehydrogenase as described by Bergmeyer *et al.*[13].

All chemicals used were of analytical grade and were obtained from either BDH or Sigma Chemical Co., St. Louis, USA. α -glycerophosphate dehydrogenase-triose phosphate isomerase was from Boehringer Corp., Mannheim, West Germany. All other auxiliary enzymes and cofactors were from Sigma, U.S.A.

Results

The activity of phosphofructokinase and pyruvate kinase in rat brain shows a regional variation (Table 1). The activities of the two enzymes in different regions of brain are in the order : cerebellum > brain stem > cerebral hemispheres. The enzymes in cerebellum is higher by 35-40% when compared with the activities in the cerebral hemispheres.

The effect of insulin-induced hypoglycemia on phosphofructokinase appears to vary for different brain regions. There was a decreased activity of phosphofructokinase in the cerebellum and the brain stem at all the time intervals after insulin adminstration (Table 1). However, the maximum effect of hypoglycemia was observed one hour after the insulin administration when cerebellar phosphofructokinase was decreased by about 30% as compared with the control.

Table 1–Activity of phosphofructokinase and pyruvate kinase during insulin-induced hypoglycemia.

Time after insulin administration (hours)[a]	Cerebral hemisphere	Cerebellum (units/g/min)	Brain stem
phosphofructokinase kinase			
Control (0)	11.5 ± 0.6	15.5 ± 0.8	13.5 ± 0.9
1	13.3 ± 0.3	11.0 ± 1.6**	11.7 ± 1.6
2	13.1 ± 1.2	13.9 ± 2.4	11.3 ± 0.8*
3.	10.4 ± 0.3	13.9 ± 1.2	8.5 ± 0.3***
Pyruvate kinase			
Control (0)	63.2 ± 2.4	89.6 ± 5.8	82.5 ± 3.0
1	46.5 ± 4.4**	69.3 ± 2.4	57.7 ± 3.4***
2	60.6 ± 3.7	87.6 ± 6.3	74.8 ± 3.3
3.	67.0 ± 7.6	87.6 ± 6.8	75.6 ± 2.1

Results are mean ± SEM of 4 or more values.
*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.
[a] The blood glucose values at 0, 1, 2, and 3 hours after insulin administration were 5.5 ± 0.2, 2.0 ± 0.2; 2.2 ± 0.2, and 2.2 ± 0.1 mM respectively.

Pyruvate kinase followed a slightly different pattern of change with insulin administration. A 20-35% decrease in activity was observed in all the three regions after one hour of insulin administration. At two and three hours, however, the enzyme activity returned to almost control levels in all three regions.

Blood glucose concetration (Table 1, legend a) decreased from 5.5 mM to about 2 mM, one hour after insulin administration, and remained more or less at this decreased level even after three hours.

Discussion

There is a regional variation in the metabolic activity of brain for the utilization of oxygen as well as glucose consumption[2], suggesting that the rate of glycolysis may vary in various brain regions. The present study shows that the activity of phosphofructokinase and pyruvate kinase is significantly higher in cerebellum than in the cerebral hemispheres, may be due to a dense packing of neuronal cells in the cerebellum and its high energy requirement[14].

The effect of altered metabolic states like hypoglycemia may also, therefore, show a differential pattern in the brain. A regionally variable effect of insulin-induced hypoglycemia on brain has been shown earlier by several authors[7, 11, 15]. Our results also show that there is a significant decrease in the activities of phosphofructokinase and pyruvate kinase in the regions of rat brain with insulin-induced hypoglycemia, though the extent and duration of decrease is different in the three regions. The glycolytic intermediates are also reported to be decreased in hypoglycemia[4, 5, 7].

The energy state of the cell in the hypoglycemia, measured as levels of ATP and phosphocreatine is reported to be little affected[14], until the glucose is well below 0.5 mM[16]. Gorell *et al.*[5] have reported that hypoglycemia stupor may be due to a specific lack of glucose

or accumulation of some metabolic products in the cerebral tissue, rather than a fuel shortage, like ATP, which remains at normal levels. According to these authors, decreased glucose utilization may be mediated by phosphofructokinase and probably caused by decreased neuronal firing. The reasons for the decreased activities of phosphofructokinase and pyruvate kinase cannot be fully explained on the basis of available evidence.

There is good evidence that hypoglycemia leads to a significant decrease in intracellular pH in the brain [16, 17] and a decrease in glycolytic flux and glucose utilization[18–20]. Since brain glycolytic enzymes are shown to be highly susceptible to decreased pH, it is likely that the lower activity of phosphofructokinase and pyruvate kinase in hypoglycemia is a consequence of the change in pH which is reflected by the decrease in brain glycolytic flux.

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Effect of cadmium on carbohydrate metabolism in freshwater teleost, *Clarias batrachus* (Linn.)

(**Key words** : cadmium toxicity/carbohydrate metabolism/*Clarias batrachus*)

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Abstract

The freshwater teleost fishes, *Clarias batrachus* were exposed to sublethal concentration *6.5 mg/L) of cadmium for 24, 48 and 96 h and the effect of this toxicant (heavy metal) on the enzymes and substrates of carbohydrate metabolism in liver, brain and gill was studied. A significant increase in glucose and lactate levels was observed while the glycogen and pyruvate contents were significantly decreased due to the toxic stress. The activities of NAD^+-LDH., ICDH and SDH were inhibited in the heavy metal exposed fish. These results suggest that cadmium blocks the oxidative metabolism in the tissues leading to an altered energy status in the fish.

Introduction

Cadmium, a non-essential heavy metal widely used in several industries is well known as one of the highly toxic environmental pollutants. Several investigators have established lethal levels of Cd compounds on fish and other aquatic animals[1-3]. The effects of Cd on histopathology[4,5], haematological parameters[6] and oxidative metabolism[7,8] of snail and fish have been studied. Limited efforts were made in unravelling the exact modulations and compensatory mechanisms in the metabolic pathways of animals against heavy metal toxicity. Hence, in the present study the effect of cadmium on the carbohydrate metabolism of a freshwater fish, *Clarias batrachus* has been investigated.

Materials and Methods

Freshwater fish, *C. batrachus* were collected from the local market around Tirupati area. The fish weighing around 25±2g and 21 cm. in length were used and were fed with groundnut cake. They were acclimatized to laboratory conditions for a week under continuous water flow.[9] The LC_{50} value (19.5 ppm) of the cadmium technical grade for 96 hrs. was determined by probit analysis[10]. The fishes were exposed to sub-lethal concentration (6.6 ppm) of cadmium for 24, 48, 72 and 96 h. Liver, brain and gill were isolated from the normal and cadmium exposed fish and were used for biochemical estimations.

Free glucose was estimated by the method of Milton and Waters[11], glycogen by the method of Carroll *et al.*[12], lactic acid according to Barker and Summerson[13] as modified by Huckabee[14] and pyruvic acid following Friedman and Hangen[15]. The activity of lactate dehydrogenase (LDH : EC 1.1.1.27) and succinate dehydrogenase (SDH : SC 1.3.99.1) were

estimated by the method of Nachlas *et al*[16] and isocitrate dehydrogenase (ICDH : EC 1.1.1.41) was estimated by the method Korenberg and Pricer.[17] The activities were expressed as μ moles of formazan formed/mg protein/h.

Results and Discussion

The data presented in Table 1 indicate that the glycogen content decreased while glucose content increased in liver, brain and gill significantly after 24, 48, 72 and 96 h of exposure to cadmium. It was reported earlier that cadmium reduced glycogen content in the fish, *T. mossambica*[6]. These studies taken together with the present data, may suggest the possible onset of an increased rate of glycogenolysis due to the heavy metal toxicity.

Further, it is clear from the data that cadmium could significantly decrease pyruvate content while markedly elevated lactate levels in all the three tissues studied. As it was

Table 1–Glycogen, glucose, pyruvate and lactate contents in the tissues of control and experimental fish. Each value is mean of 6 individual observations

Parameter	Name of the tissues	Control	Exposed			
			24 h	48 h	72 h	96 h
Glycogen (μg/100 mg wet wt.)	Liver	550.02 ± 0.24	427.14 ± 0.02	394.04 ± 0.11	325.22 ± 0.001	284.00± 0.82
		–	–22.34	–28.35	–40.87	–48.36
	Brain	130.41 ± 0.38	109.02 ± 0.34	81.34 ± 0.38	70.72 ± 0.44	61.04 ± 0.11
		–	–16.40	–37.62	–45.77	–53.19
	Gill	99.14 ± 0.98	81.44 ± 0.31	67.01 ± 0.74	51.71 ± 0.38	41.82 ± 0.24
		–	–17.85	–32.40	–47.84	–57.81
Glucose (μg/100 mg wet wt.)	Liver	128.32 ± 0.21	144.38 ± 0.44	154.08 ± 0.38	169.71 ± 0.34	177.11 ± 0.11
		–	–12.51	–20.07	–32.25	–38.02
	Brain	28.91 ± 0.24	39.00 ± 0.34	48.18 ± 0.71	59.18 ± 0.79	65.09 ± 0.41
		–	–34.90	–66.65	–73.57	–76.72
	Gill	39.64 ± 0.19	47.64 ± 0.82	52.14 ± 0.38	69.28 ± 0.24	76.14 ± 0.82
		–	–20.18	–31.53	–49.54	–66.85
Pyruvate (μg/100 mg wet wt.)	Liver	58.52 ± 0.54	41.82 ± 0.31	39.01 ± 0.31	20.61 ± 0.68	15.78 ± 0.82
		–	–18.28	–33.33	–47.69	–55.94
	Brain	19.98 ± 0.93	15.72 ± 0.14	10.78 ± 0.34	7.68 ± 0.67	4.42 ± 0.38
		–	–11.31	–21.32	–41.04	–62.86
	Gill	57.41 ± 0.41	44.78 ± 0.71	38.82 ± 0.38	29.74 ± 0.48	22.98 ± 0.38
		–	–13.30	–27.15	–49.93	–54.74
Lactate (μg/100 mg. wet wt.)	Liver	0.15 ± 0.31	0.20 ± 0.74	0.37 ± 0.54	0.40 ± 0.73	0.58 ± 0.32
		–	33.33	80.00	86.66	100.00
	Brain	0.23 ± 0.11	0.31 ± 0.42	0.43 ± 0.62	0.59 ± 0.88	0.64 ± 0.92
			34.78	43.47	69.56	91.36
	Gill	0.14 ± 0.72	0.18 ± 0.04	0.20 ± 0.72	0.29 ± 0.38	0.36 ± 0.11
		–	28.57	49.83	107.14	157.11

suggested that heavy metals could induce hypoxic or anoxic conditions[18], the observed increase in lactate content could be due to an enhanced anaerobiosis in these tissues due to toxic stress[19].

Table 2 shows that changes in the activities of NAD^+–LDH, ICDH and SDH. LDH activity was significantly decreased in liver, gill and brain tissues of fish. The decrease in NAD^+ dependent LDH may lead to a metabolic shift from aerobiosis to anaerobiosis during cadmium exposure. The decrease in ICDH and SDH activities may be due to depressed energy metabolism during cadmium intoxication. Cd has been found to be inhibitory to many enzymes such as SDH, ICDH and alkaline phosphatases in fish, *Pseudopleuronertes americanus*[20]. The lowere energy status might have compensatory influence in the maintenance of ICDH activity. However, a significant inhibition of SDH activity was observed in all the three tissues at 24, 48, 72 and 96 h. suggesting an overall reduction in the energy output. Supporting evidence for the lower energy status shown from the observations suggests that the pesticides and heavy metals exert their toxic action by impairing the mitochondrial function[8].

Table 2–Activities of NAD^+ –LDH, ICDH and SDH in the tissues of control and experimental fish. Each value is mean ± SD of 6 individual observations.

Parameter	Name of the tissues	Control	Exposed			
			24 h	48 h	72 h	96 h
NAD^+–LDH	Liver	1.48 ± 0.03	1.31 ± 0.38	1.11 ± 0.98	1.04 ± 0.72	0.87 ± 0.81
(μ mol	% change	–	–11.48	–25.00	–29.72	–41.21
formazan	Brain	0.39 ± 0.07	0.32 ± 0.17	0.29 ± 0.34	0.22 ± 0.98	0.19 ± 0.11
formed/mg	% change	–	–17.94	–25.64	–43.58	–51.82
protein/h)	Gill	0.46 ± 0.09	0.39 ± 0.18	0.32 ± 0.67	0.27 ± 0.82	0.20 ± 0.55
	% change	–	–15.21	–31.43	–41.30	–56.52
ICDH	Liver	0.49 ± 0.114	0.42 ± 0.08	0.24 ± 0.08	0.30 ± 0.63	0.22 ± 0.004
(μ mol	% chnage	–	–14.28	–30.61	–38.77	–55.10
formazan	Brain	0.18 ± 0.01	0.15 ± 0.18	0.10 ± 0.018	0.08 ± 0.002	0.05 ± 0.34
formed/mg.	% change	–	–16.66	–44.44	–55.55	–72.22
Protein/h)	Gill	0.053 ± 0.032	0.047 ± 0.18	0.038 ± 0.001	0.029 ± 0.11	0.021 ± 0.38
	% change	–	–11.32	–28.30	–45.28	–60.37
SDH	Liver	4.78 ± 0.11	3.97 ± 0.82	3.84 ± 0.11	3..68 ± 0.78	3.42 ± 0.14
(μ mol	% change	–	–16.94	–19.66	–23.01	–28.45
formazan	Brain	1.89 ± 0.06	1.72 ± 0.18	1.63 ± 0.82	1.51 ± 0.74	1.33 ± 0.28
formed/mg.	% change	–	–8.99	–13.75	–20.10	–29.62
Protein/h	Gill	0.89 ± 0.04	0.78 ± 0.38	0.64 ± 0.78	0.52 ± 0.64	0.31 ± 0.88
	% change	–	–12.35	–28.08	–41.57	–65.16

Increased glycogen mobilization, enhanced pyruvate conversion to lactate and inhibition of citric acid cycle enzymes clearly suggest increased oxidation of glucose through the anaerobic glycolytic pathway to provide energy for the fish. This is associated with significant reduction in the mitochondrial oxidative metabolism due to the derangement of mitochondrial function directly or indirectly by the pollutant, cadmium.

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Effect of herbicides on the uptake potential of nitrogen, phosphorus and potassium for aquatic weeds from farm ponds

(Key words: aquatic weed control/removal of essential nutrients/nitrogen/phosphorus/potassium)

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Abstract

The present study was carried out at the research farm, Institute of Agricultural Sciences, Banaras Hindu University, during the year 1981-82 and 1982-83 in a randomised block design to evaluate the efficacy of three herbicides in containing nutrient losses by acquatic weeds. Significant depletion in nitrogen, phosphorus and potassium removal was observed after herbicidal treatment in all the seasons. Paraquat @ 1.0 litre/ha was the most effective weedicide followed by 2, 4–D and dalapon. In control condition, aquatic weeds removed 51.88 kg nitrogen, 5.97 kg phosphorus and 49. 66 kg potassium per ha. Summer season was found to be best in containing the uptake potential of essential plant nutrients by aquatic weeds.

Introduction

Excessive growth of aquatic weeds is undesirable for irrigation, livestock and fish. It creates serious problems in different water bodies. These weeds lower the nutrient status of pond soil and water by absorbing nutrients for their luxuriant growth. The uptake of essential plant nutrients by water weeds is a serious concern to the researchers. Steward[1] showed that certain species of the aquatic plants can remove nitrogen from 86 to 5896 kg and phosphorus 9 to 590 kg/ha. McCord and Loycano[2] studied the removal of nutrients from a pond through *Eleocharis dulcis* which removed 108.06 kg nitrogen, 6.9 kg calcium and 37.46 kg magnesium in 201 day growing season. However, the literature on removal of plant nutrients by aquatic weeds from an ecosystem is scanty. Further inquest is, therefore, necessary to assess the quantum of nutrient losses and to evaluate different herbicides for their efficacy in preventing their depletion from ponds.

Material and Methods

The present investigation was carried out at the Research Farm, Institute of Agricultural Sciences, Hindu University, during 1981-82 and 1982-83 on farm ponds. The experiment was planned in randomised block design with three replications. The seasons were also recognised as experimental variables. As such there were twelve treatment combinations (4 herbicidal treatment x 3 seasons) in the experimental project. The number of samples drawn in each

sampling has been taken as replication. Analysis of variance was done to test the significance of differences between means of the treatments as per Cochran and Cox[3].

In the beginning of three seasons, the herbicides were sprayed on the vegetative foliage on water area basis. Sodium salt of dalapon (2, 2, dichloropropionic acid) 15.0 kg/ha; sodium salt of 2, 4–dichlorophenoxy acetic acid (2, 4–D) 1.5 kg/ha and paraquat (1, 1–dimethyl–4, 4–bipyridinium ion) 1.0 litre/ha were sprayed in three different ponds while one pond was taken as control. The area of each pond was approximately 0.26 ha and average water depth varied in different seasons from 1.25 m to 3.50 m.

Sampling was done at 7 and 49 days after spraying to estimate the nutrient losses with the help of quadrate sampler (50 cm x 50 cm). The samples were analysed for nitrogen, phosphorus and potassium as per the methods of Jackson[4]. The total removal of nutrients by weeds was calculated by multiplying the dry matter of weeds with their respective per cent concentration.

Results and Discussion

The important weed flora present in the selected farm ponds were identified. There were some emergent weeds (*Eleocharis plantaginea, Zizania aquatica, Paspalum scrobiculatum* and *Nymphaea stellata*) and floating weed (*Eichhornia crassipes*) present in the farm ponds. These weeds were also present after 7 and 49 days of herbicidal applications except in summer season when *Zizania aquatica* and *Nymphaea stellata* were absent.

The result of the investigation are presented in Table1. The effect of herbicides and seasons is discussed separately.

Effect of herbicides : Significant reduction of nitrogen, phosphorus and potassium removal was observed after herbicidal application at both the stages as compared to control. Paraquat application @ 1.0 litre/ha was most effective for reducing the depletion of all the three elements at both the stages of observation. It was followed by 2, 4–D and dalapon at 7 days after spraying and dalapon and 2, 4–D at 49 days after spraying during both the years of observation (Table 1). The quantum of nutrient removal differed significantly between paraquat and the other two herbicides but the differences were not always significant between dalapon and 2, 4–D. The removal of nitrogen, phosphorus and potassium by the aquatic weeds in control was 51.88 kg/ha, 5.97 kg/ha and 49.66 kg /ha respectively.

Paraquat was found to be most suitable in containing nutrient removal by weeds during both the years of observation. A drastic reduction in dry matter of weeds may account for this which had occurred due to high phytotoxic effect of this herbicide resulting in quick desiccation of weeds. The light reaction in photosynthesis which involves the absorption of light by chlorophyll and transformation of light energy into chemical energy via a series of electron transfer reactions leading to the formulation of reduced pyridine nucleotide (NADPH) and adenosine triphosphate (ATP) might have been affected.

Table 1–Effect of herbicides and seasons on uptake potential of nitrogen, phosphorus and potassium (kg/ha) by aquatic weeds.

Treatments	Days after spraying											
	1981-82						1982-83					
	Nitrogen		Phosphorus		Potassium		Nitrogen		Phosphorus		Potassium	
	7	49	7	49	7	49	7	49	7	49	7	49
Herbicides												
Dalapon	15.313	6.418	1.713	0.739	15.406	8.190	13.128	6.271	1.472	0.727	13.237	7.799
2, 4–D	11.276	6.620	1.253	0.745	14.321	9.025	9.463	6.447	1.121	0.736	9.577	8.826
Paraquat	3.309	1.409	0.359	0.143	4.151	1.970	2.605	1.301	0.286	0.134	3.391	1.862
Control	46.732	51.505	5.274	5.846	44.819	48.878	47.821	51.888	5.483	5.971	46.485	49.665
S Em ±	0.8825	0.9413	0.0950	0.0896	0.9139	0.8254	1.4601	1.4269	0.2411	0.3189	1.2446	1.2863
C.D. (0.05)	2.5885	2.4677	0.2786	0.2629	2.6806	2.4209	4.2827	4.1853	0.7071	0.9354	3.6505	3.7728
Seasons												
Summer	10.860	8.796	1.274	1.027	13.865	11.296	11.628	12.115	1.334	1.392	14.551	14.956
Monsoon	19.706	20.113	2.193	2.256	19.683	20.023	19.686	19.518	2.239	2.245	19.567	19.449
Winter	26.906	20.555	2.982	2.323	25.475	19.730	23.449	17.797	2.699	2.039	22.773	16.708
SEm ±	0.7643	0.7286	0.0822	0.0776	0.7915	0.7148	1.9645	1.2357	0.2088	0.2762	1.0778	1.1139
C.D. (0.05)	2.2417	2.1371	0.2413	0.2277	2.3215	2.0966	3.7089	3.6246	0.6124	N.S.	3.1615	3.2674

Effect of seasons : Seasonal differences had marked effect on depletion of all the three nutrients following the application of herbicides. The lowest depletion of nitrogen, phosphorus and potassium was recorded in summer season in both the years (Table 1). However, it generally increased through the monsoon and winter seasons at both the stages. Significant differences between summer and mosnoon and summer and winter seasons were observed. But there was no significant variation among seasons at 49 days in case of phosphorus removal.

Summer season was found to be most effective in restricting the uptake of essential nutrients by weeds from the ponds partly because of partial chlorosis and partly as a result of inefficient photosynthetic activities of weeds due to decreased chlorophyll status of the net assimilatory area. The amount of chlorophyll has a direct connotation in relation to photosynthetic activity of weeds. Besides, the increased entry of herbicides, due to greater stomatal opening during summer, might have been responsible for further increase in rate of respiration and transpiration resulting into faster metabolic activity and greater utilization of source energy than its production causing death of plants[5].

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Population dynamics of rotifer fauna from two tropical ponds of Aligarh, India

(Key words : rotifer/eutrophication/indicators)

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Abstract

Studies were conducted on rotifer population along with physico-chemical parameters of two tropical ponds at Aligarh. Altogether ten species of rotifers were identified and their abundance was noted. Two peaks of production were noted in Nagla pond and one in ITI pond. *Brachionus calyciflorus* and *B. plicatilis* were found to be the main contributors in both the ponds. An inverse relationship was noted with total alkalinity and transparency. The species *Brachionus calyciflorus* and *B. plicatilis* have shown much resistance to adverse environmental conditions prevailing in the pond.

Introduction

The quality of environment is degrading rapidly due to various types of pollutants and contaminants released from different sources. The study of a natural population in its environment requires a careful evaluation of the interrelationships between the population and different biological, chemical and physical parameters of the environment.

Among zooplankton population, rotifers are apparently the most sensitive indicators of water quality and their presence may be used as a reference of the physico-chemical characteristics of their habitat. In the present investigation, rotifers were collected from two different ponds and compared along with some physico-chemical conditions.

Materials and Methods

The studies were carried out from January, 1986 to December, 1986 in two freshwater polluted ponds known as Nagla and I.T.I. ponds. The I.T.I. pond is a sewage-fed pond with its depth varying from 3 to 7 feet during different seasons, whereas Nagla pond receives detergents and waste matters released from the factories situated near it. The portion of the Nagla pond which receives industrial waste, is much polluted and the water is always found to be blackish in colour. Its mean depth varied from 3 to 5 feet during different seasons.

Monthly planktons were collected from two different points by filtering 100/1 of water through a plankton net (bolting silk No. 25, mesh size 64 μ) and were preserved in 5% formaline. Countings were made in a Sedgewick Rafter counting cell. The methods used for the physico-chemical analysis of water and qualitative as well as quantitative estimations of the plankton were followed according to APHA[1].

Results and Discussion

Results for the physico-chemical parameters have been illustrated in Figs. 1 & 2.

The average mean values of surface water temperature ranged from 12.0 to 33.0°C (I.T.I.) and 13.7 to 31.0°C (Nagla). The maximum Secchi disc transparencies were noted in March, 1986 (both the ponds) and minimum in the month of July (I.T.I. ponds) and August, 1986 (Nagla pond).

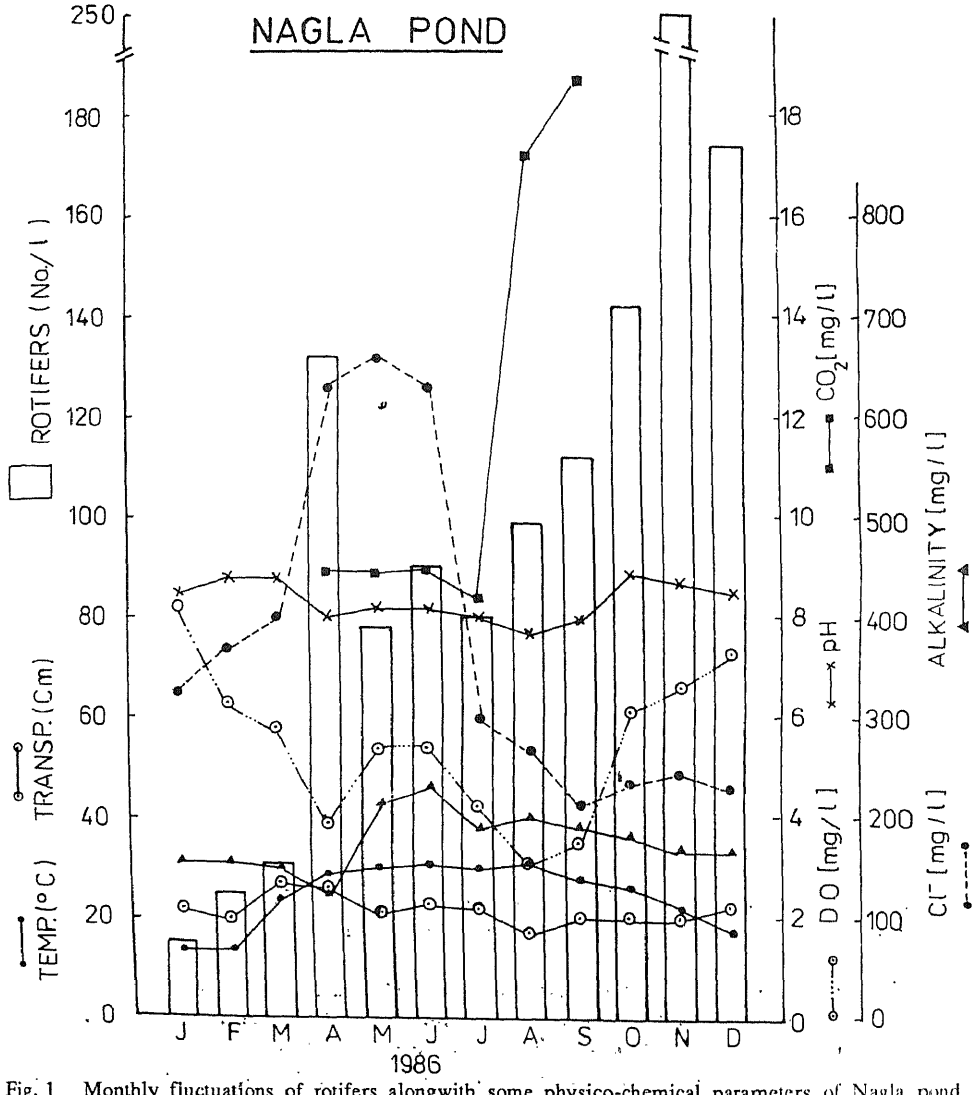

Fig. 1 Monthly fluctuations of rotifers alongwith some physico-chemical parameters of Nagla pond during 1986.

The dissolved oxygen content of the ponds was found to range from 4.1 to 14.2 mg/l (I.T.I. pond) and 3.10 to 8.25 mg/l (Nagla pond). Higher concentrations were observed during December, January and Febraury, 1986 in both the ponds.

The waters were always found alkaline throughout the year in both the ponds. The pH ranged from 7.9 to 9.5 in I.T.I. pond and 7.7 to 8.9 in Nagla pond. The minimum values of pH coincided with monsoon months of this region. Free CO_2 was recorded during April to September, 1986 in Nagla pond only.

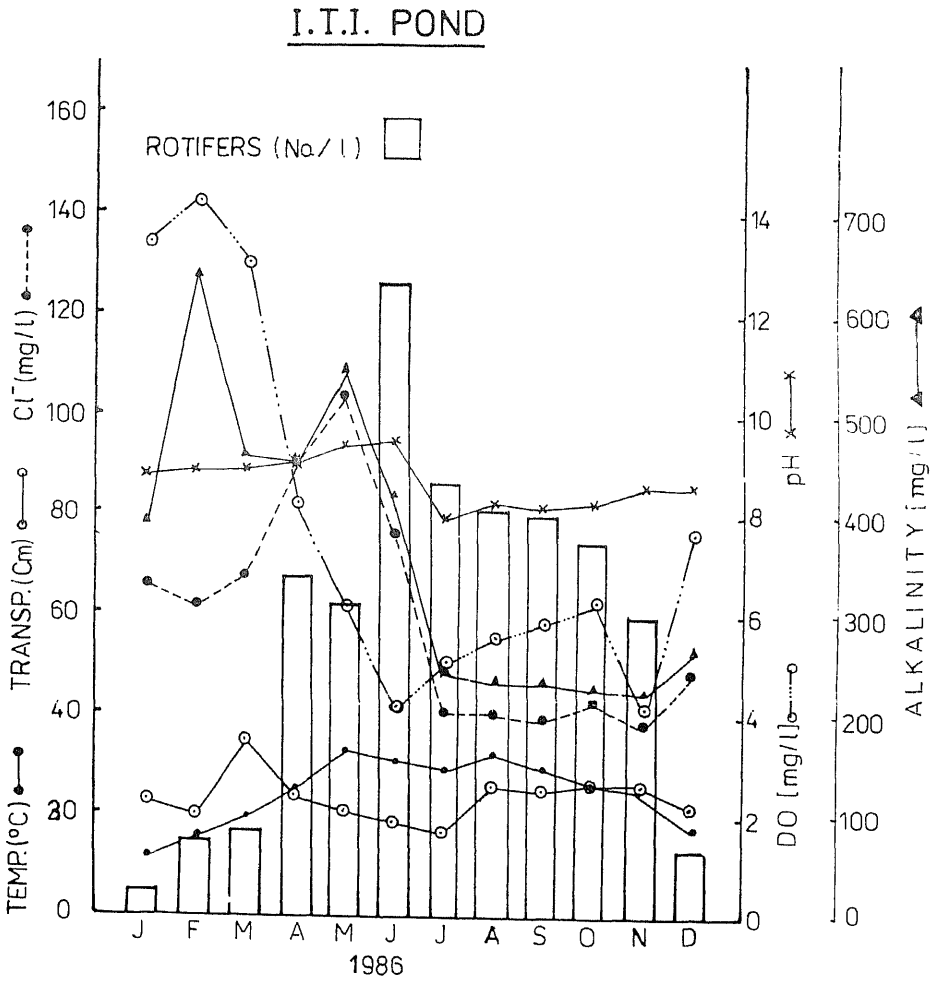

Fig. 2 Monthly fluctuations of rotifers alongwith some physico-chemical parameters of I.T.I. pond during 1986.

Qualitiative and quantitiative counts of zooplankton were carried out and the results have been expressed numerically in units per litre. Among zooplankton, rotifers have been given special emphasis showing two peaks in Nagla pond (April & November) and one peak in I.T.I. pond (June) during the tenure of investigations. Ten species of rotifers belong to four genera, *Brachionus, Filinia, Testudinella* and *Keratella* in descending order of their abundance in Naglapond whereas in I.T.I. pond only nine species belonging to the same four genera in descending order of their abundance were noted (Tables 1 and 2).

The major contributors in both the peaks of Nagla pond were *Brachionus calyciflorus* and *B, plicatilis,* which were noted throughout the year but in I.T.I. pond the peak was shared by *Brachionus calyciflorus* and *B, plicatilis, Keratella canadensis* and *Testudinella* sp. (Tables 1 and 2). All the four species of genus *Brachionus,* namely, *B. calyciflorus, B. plicatilis, B. bidentata* and *B. quadridentata* and *Filinia terminalis* were always found to be present in I.T.I. pond, whereas in Nagla pond, only two species of genus *Brachionus* (*Brachionus calyciflorus* and *B, plicatilis*) were observed. Chourasia[2] has also reported that certain forms of rotifers were observed throughout the year, while others exhibited appreciable numerical fluctuations and resulted in distinct pulses in the total rotifer population.

In winter, ponds showed a substantial decrease in water temperature which coincided with lowest population densities of rotifers in both the ponds. It was mainly due to disappearance of *Brachionus bidentata, B. angularis, Keratella canadensis, K. valga* and *Testudinella* sp. Reappearance of the aforesaid species from March onward was mainly caused on getting favourable climatic conditions for their reproduction and development (Fig. 1 and 2).

The rotifier population of these ponds showed no direct relationship with pH. Pejler[3] has reported that rotifers are pH insensitive but Arora[4] has experimentally shown that most rotifer species are sensitive to pH fluctuations. Van Oye[5] considered that it is not possible to draw conclusions from pH, because in natural conditions it never changes alone. Similarly, Edmondson[6] suggested that more than one variable may be involved in producing an apparent limitation to a certain range of pH.

The total alkalinity and transparency showed inverse relationships with total rotifer population in both the ponds (Fig. 1 and 2). These parameters were higher in I.T.I. pond as compared to Nagla pond. Nagla pond receives industrial wastes from the adjoining factories which makes the water brownish in color and reduces its alkalinity as well as transparency. Even though the data show an inverse relationship with alkalinity and transparency, the actual population dynamics may be due to cumulative effect or due to a combination of several environmental factors.

Dissolved oxygen values were quite high throughout the year in both the ponds showing no direct effect upon the rotifer population. Much higher concentration in I.T.I. as compared to Nagla pond was mainly due to luxuriant growth of aquatic plants near the shore line.

The chloride ion was much more abundant in Nagla pond. It showed no pronounced effect on the rotifer population of the ponds.

Table 1–Seasonal variations in rotifer population in Nagla pond during 1986 (Nos./1).

Rotifers	Months											
	Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
Brachionus calyciflorus	2	5	7	10	12	17	15	13	12	35	70	76
B. bidentata	–	–	–	6	14	7	8	2	10	5	6	–
B. plicatilis	13	16	20	93	25	24	15	17	20	23	71	72
B. havanaensis	–	–	–	–	2	5	6	6	13	14	8	3
B. quadridentata	–	1	1	8	4	12	3	5	7	5	25	20
B. angularis	–	–	–	–	–	–	1	3	2	5	1	–
Keratella canadensis	–	–	–	–	–	4	7	5	12	14	21	–
Filinia terminalis	–	1	2	1	3	5	8	6	9	10	8	3
F. longiseta	–	2	1	8	7	3	2	6	7	9	15	–
Testudinella sp.	–	–	–	6	11	13	15	16	20	22	25	–
Total	15	25	31	132	78	90	80	99	112	142	250	174

Table 2–Seasonal variations in rotifer population in I.T.I. pond during 1986 (Nos./l).

Rotifers	Months											
	Jan.	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.
Brachionus calyciflorus	2	4	6	17	19	16	16	15	14	13	9	3
B. bidentata	–	1	1	3	2	4	13	9	2	10	7	–
B. plicatilis	–	3	4	17	6	15	14	20	22	13	14	10
B. quadridentata	1	2	3	1	3	2	1	1	4	3	2	1
Keratella canadensis	–	–	–	7	10	17	3	2	4	2	1	–
K. cochlearis	–	–	–	–	4	9	3	1	5	–	–	–
K. valga	–	–	–	1	3	4	1	5	6	9	8	–
Filinia terminalis	2	5	3	14	12	10	21	18	3	4	15	–
Testudinella sp.	–	–	–	7	3	40	15	10	20	21	4	–
Total	5	15	17	67	62	126	86	81	80	75	60	14

After going through the above discussion we can conclude that rotifers are apparently the most sensitive indicators of water quality and trophic conditions of the habitat. The species *Brachionus calyciflorus* and *B, plicatilis,* showed much resistance to adverse environmental conditions. Their numerical strength can be used as a basis for evaluating the conditions of the water bodies.

Acknowledgement

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Ovarian interstitial gland cells of teleosts, *Puntius stigma* and *Mystus bleekeri*

(Key words : interstitial gland cells/teleosts/steriods)

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Abstract

Origin and chemical nature of ovarian interstitial gland cells (IGC) of teleost, *Puntius stigma* and *Mystus bleekeri* have been studied. The IGC arise from amongst the remains of atretic and postovulatory follicles of *Puntius* and from stromal tissue of *Mystus*, and include proteins, lipids (neutral lipids) and 3β-hydroxysteroid dehydrogenase.

Introduction

Interestitial gland cells (IGC) of mammalian ovary have been well studied[1]. In the submammalian vertebrate ovary, however, the very existence of the IGC has been doubtful, primarily because of their transitory nature[2]. In the teleostean catfish ovary the IGC reportedly orginate from the thecal elements of the large previtellogenic atretic follicles and contain enzymes concerned with steroid synthesis[3]. In the present communication an attempt has been made to study the origin and chemical nature of the IGC in the ovary of two locally available fresh water teleosts, *Puntius stigma* and *Mystus bleekeri.*

Materials and Methods

Fish after collection was dissected immediately and ovary was fixed in Bouin's 10% neutral formalin and formol-calcium. As usual, paraffin sections were used for histological and cytochemical preparations for locating proteins and carbohydrates, while cryocut (American Opticals) frozen sections were used for staining of lipids and enzymes. Masson's trichrome was used for histological staining. For cytochemical staining, periodic acid-Schiff (PAS) and alcian blue (AB) were used for polysaccharides, mercuric bromophenol blue (HgBPB) and coupled tetrazonium (CTZ) for proteins, Sudan black B (SBB) for lipids, Sudan III + IV for neutral lipids, acid haematein (AH) for phospholipids, and Wattenberg's method for 3β-hydroxysteroid dehydrogenase (HDH) as described in Pearse[4].

Observations

Interstitial gland cells, which are almost round in shape and possess small darkly-stained eccentrically placed nuclei, visibly differentiate from amongst the hyperactive follicle and thecal cells of the atretic (Fig.1) and postovulatory follicles of *Puntius stigma* (Fig. 2) and in clusters from the stromal tissue of *Mystus bleekeri* (Fig. 3).

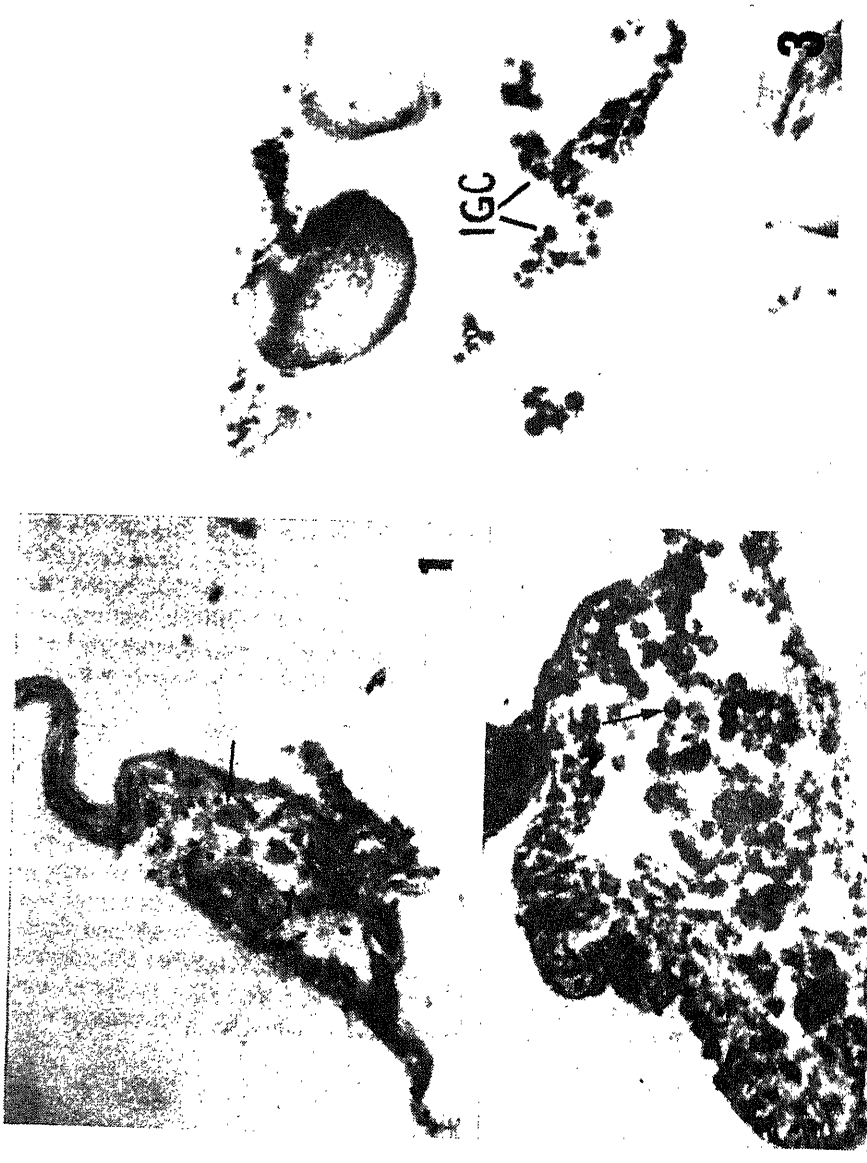

Fig. 1 Atretic follicle of *Puntius stigma* with differentiating interstitial gland cells (arrow). HgBPB x 400.
Fig. 2 Postovulatory follicle of *Puntius stigma* with differentiating interstitial gland cells (arrow). CTZ x 400.
Fig. 3 Clusters of interstitial gland cells (IGC) differentiating from the stromal tissue of *Mystus bleekeri*. Masson's trichrome x 400.

Cytochemically, the interstitial cells in both the fish react positively for proteins, general and neutral lipids, and HSDH enzyme, while their PAS–and AB–negative character reveals absence of carbohydrates, and AH–negative character absence of phospholipids.

Discussion

There is difference of opinion as to the occurrence of interstitial gland cells in the fish ovary. Hoar[5] stated that the fish ovary does not contain interstitial tissue comparable in development and histochemistry to the interstitial (Leydig) cells of the testis, while presence as well as steroidogenic activity of the IGC have been demonstrated in the ovary of *Mystus cavasius*[3] and *Monopterus albus*[6]. In the present case, the IGC could clearly be located in the ovary of both *Puntius stigma* and *Mystus bleekeri.*

In most of the articles dealing with follicular atresia in different vertebrate classes, investigators have generally noted that, after the completion of atresia, some hypertrophied thecal cells remain in the ovarian stroma, but only a few workers stated these cells might contribute to the IGC[7]. In the present study, while their differentiation could not be observed in the atretic follicles of *Mystus,* the gland cells clearly show their appearance in those of *Puntius.* Besides, the gland cells have also been reported to arise from regular connective tissue of stroma in submammalian vertebrate ovaries[7] as observed presently in *Mystus.* Their differentiation from amongst the cells of postovulatory follicles as shown in *Puntius* has, however, not been reported hitherto in teleosts.

Cytochemical reactions in the present study reveal the presence of lipids (neutral lipids) and 3β-HSDH enzyme, indicating the steroidogenic nature of the IGC in both *Puntius* and *Mystus.* In a few teleosts, such as *Mystus cavasius*[3] and *Brachydanio rerio*[8] also it has been shown convincingly that the IGC possess steroidogenic potentiality as revealed by histochemical tests. Even ultrastructural characteristics of steroidogenic cells, that is, presence of large mitochondria with tubular cristae and agranular endoplasmic reticulum as observed in the testicular IGC of teleost fishes[7] have also been reported in the ovarian IGC of *Brachydanio*[8].

In view of these reports it appears possible to visualise that the ovarian IGC of teleosts also secrete steroid hormones as in higher vertebrates. In mammals, the major steroids secreted by the IGC of the ovarian stroma include progestins, androgens and estrogens depending on the species as wll as on the physiological situations[7]. It can be presumed that similar steroid hormones, which play an important role in the reproductive physiology, might also be secreted by the IGC of teleosts including *Puntius* and *Mystus.* This assumption, however, should be confirmed by further investigations.

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Gross chemical composition of different stages of *Antheraea mylitta* (Saturniidae : Lepidoptera)

(Key words : *Antheraea mylitta*/chemical composition/water/lipids/protein/carbohydrate/ash)

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Abstract

A study of chemical composition of *Antheraea mylitta* in different stages has revealed that water and carbohydrate contents increase during larval development. They show a drop in early pupa, increase in middle and late pupa and a drop again in the adult stage. Protein content decreases from about 63% to 59% during larval development, remains steady during pupal stage and increases again to a level of 61% in the adult. Lipid content increases from around 19% to 24% in larval life and then decreases to 19% in the adult as a result of utilization for energy requirements. The significance of changes in chemical composition during life is discussed.

Introduction

The life-history of the tasar silk insect, *Antheraea mylitta,* includes the egg, five larval instars, pupa and adult stage. Of these, the larvae are voracious feeders and they exhibit tremendous growth. The pupa and the adults do not feed, and energy for all vital activites, viz. histogenesis, organogenesis, metamorphosis and reproduction is derived from the great amount of food reserve accumulated in the larval stage. It is obvious that biochemical parameters must be undergoing tremendous change in the course of development, metamorphosis and reproduction. For a better understanding of the biology of the tasar silk insect, it would be necessary to make a study of gross chemical composition of the insect in all stages of its life and this should be followed by a detailed study for information on biochemical transformations in relation to metabolic activities. The present communication is a report on the gross chemical composition of *Antheraea mylitta* in the larval, pupal and adult life with reference to water, lipid, protein, carbohydrate and ash contents.

Materials and Methods

Cocoons of *A. mylitta* were obtained from Adivasi rearers of Chaibasa (South Bihar) in August and maintained in the laboratory according to the method described by Krishnaswamy *et al.*[1] and Jolly[2]. Following emergence and copulation, the females laid eggs. The larvae hatching out of the eggs were reared on mature leaves of pruned sal trees[3]. Pupae were harvested and maintained in the laboratory till the emergence of adults. Water, lipid and protein contents of all the five larval instars, pupa (early, middle and late stages) and adults (male and female) were determined as described below :

$$1.\ \text{Water content (\%)} = \frac{\text{Fresh wt.} - \text{dry wt.}}{\text{Fresh wt.}} \times 100$$

2. Lipids from a weighed quantity of powdered dried insect were extracted with petroleum ether. Following evaporation of petroleum ether, the dried residue was weighed. Lipid content (% dry wt.) was calculated as :

$$\frac{\text{dry wt. of extracted lipid}}{\text{Initial dry wt. of insect}} \times 100.$$

3. Protein was estimated by determining the % nitrogen content by Duma's method[4] multiplying it with 6.25. Although this method does not permit accurate estimation of protein content, hence not in use these days, but it does give an indication of the trend of change in protein content in different stages of the life of the insect.

4. Ash content was estimated by heating a weighed dried insect in a crucible over a burner for several hours. After allowing it to cool down, the residue was weighed again. Ash content

$$= \frac{\text{Weight of ash}}{\text{Initial dry wt. of insect}} \times 100.$$

5. Carbohydrate + other materials, if any = dry wt. of insect – weight of (protein + lipid + ash).

Estimations were made on day 3, 8, 13, 18 and 25 for larval instars I to V respectively, day 30, 50 and 70 for early, middle and late pupal stages and on day 80 for adults. The quantitative data collected for all stages of the insect were recorded and subjected to appropriate statistical analysis involving correlation, regression, t-test and analysis of variance.

Results

The data on chemical composition of different stages of *A. mylitta* are recorded in Table 1 and the pattern of changes during growth and metamorphosis are presented in Fig. 1. On absolute weight basis, all constituents (water, lipid, protein, carbohydrate and ash) are observed to increase in amount with increase in weight of the larva from instar I to V, but comparision of contents on % basis, shows a different picture.

Water content of the larva increases from 75.83% in the I instar to 79.69% in the V instar. It drops down to 76% in early pupa and rises again to 77.77% in the late pupa. Statistically, this rise is observed to be highly significant ($P < 0.001$; C.D.= 0.23). This is followed by a decrease to 74.6% in the adult stage. The female moth has significantly ($P < 0.001$) higher water content (75.93%) than that of male (73.33%).

The lipid content (dry wt. basis) shows a progressive increase form 19.4% in the I instar larva to 23.79% in the V instar. This is followed by a sharp decrease from 23.2% to 18–19% from early pupa to adult. The female moth has a significantly ($P < 0.01$) higher lipid content (19.2%) than male (18.34%).

Protein content (dry wt. basis) decreases from 63.69% in the I instar larva to 59.0% in the fifth. It remains steady during the pupal phase (around 59%) and then significantly goes up ($P < 0.001$) to 60.7% in the adult there being no significant difference between male and female(Table 1).

Carbohydrate content (dry wt. basis) progressively increases during larval phase from 7.6 (I instar) to 10.16% (V instar), drops down to 9% in early pupa and then shows a significant ($P < 0.001$) rise to 10.48% in the late pupa. There is no significant difference in the carbohydrate content of late pupa and male adult (19.16%), but the female moth has a significantly ($P < 0.01$) lower level (9.45%).

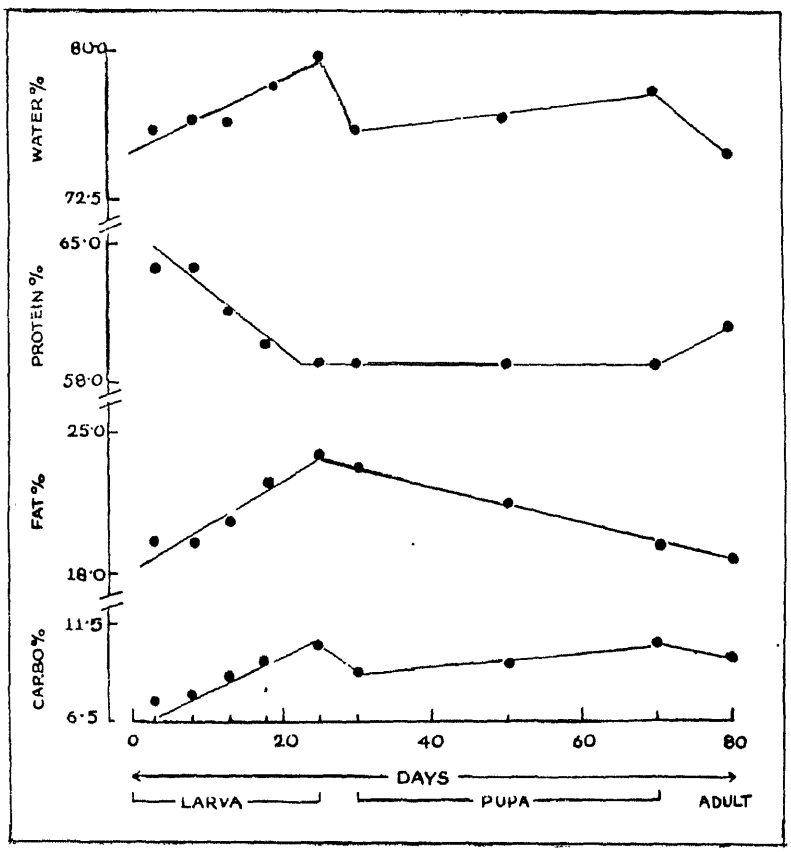

Fig. 1 Gross chemical composition of *Antheraea mylitta* in different stages of life (Carbohydrate, Fat & Protein contents shown on % Dry Weight basis).

Table 1–Chemical composition of different stages of *A mylitta*. Values are mean ± S.E. of 4 observations. Figures in parenthesis in the column of adult indicate mean of the values for male and female.

Constituent	LARVA					PUPA			ADULT	
	I	II	III	IV	V	Early	Middle	Late	Male	Female
Water	75.83 ± 0.16	76.27 ± 0.09	76.25 ± 0.15	78.31 ± 0.18	79.69 ± 0.05	76.01 ± 0.04	76.57 ±0.16	77.77 ± 0.08	73.33 ± 0.16 (74.63)	75.93 ± 0.03
Lipid (% dry wt.)	19.45 ± 0.04	19.47 ± 0.03	20.69 ± 0.30	22.63 ± 0.10	23.79 ± 0.10	23.21 ± 0.10	21.47 ± 0.07	19.54 ± 0.16	18.34 ± 0.20 (18.84)	19.20 ± 0.05
Protein (% dry wt.)	63.69 ± 0.17	63.63 ± 0.04	61.50 ± 0.09	60.08 ± 0.21	59.07 ± 0.47	58.99 ± 0.16	59.14 ± 0.04	59.21 ± 0.06	60.55 ± 0.13 (60.72)	60.86 ± 0.10
Carbohydrate (% dry wt.)	7.60 ± 0.14	7.79 ± 0.09	8.84 ± 0.11	9.54 ± 0.09	10.16 ± 0.05	9.09 ± 0.15	9.50 ± 0.04	10.48 ± 0.11	10.16 ±0.19 (9.80)	9.45 ± 0.13
Ash (% dry wt.)	9.24 ± 0.05	9.07 ± 0.03	9.00 ± 0.11	7.87 ± 0.21	7.00 ± 0.07	8.65 ± 0.13	9.90 ± 0.45	10.12 ± 0.03	10.44 ± 0.07 (19.60)	10.76 ± .02

Discussion

The developing insect represents a dynamic system and changes in chemical composition are related to morphogenetic and physiological events.[5,6]. From Table 1 and Fig. 1, it would be clear that profound changes occur in the chemical composition of *A. mylitta* during postembryonic development. These changes conform to the general pattern described[7–9] in relation to holometabolous insects.

In the larval phase of *A. mylitta*, fat and carbohydrate contents increase steadily so that they may be used for energy requirements during non-feeding pupal and adult life for metamorphosis and reproductive activities respectively. The larvae feed voraciously on the leaves of host plants (sal, arjuna). The leaves provide carbohydrate, but very low amount of lipid material. Heavy fat deposit in larvae results from conversion of carbohydrate to fat – a common phenomenon in the larvae of endopterygotes[10]. The mature larva of *Bombyx mori* has twice as much fat as in the leaves it has consumed and the greater part of this is neutral fat formed from carbohydrate as quoted by Wigglesworth[11].

In the pupal phase, lipid content decreases and carbohydrate content increases. This is suggestive of utilisation of fat for energy requirements and also conversion of fat to

carbohydrate. Reports of this kind are available in relation to other species of silkworms. In *Antheraea pernyi*, carbohydrate content increases from egg to pupa and declines in the development of the adult[12]. During pupal development, *Bombyx mori* uses fat for nearly 50% of its total energy and the remainder is covered chiefly by carbohydrates[10]. The respiratory quotient at the time of spinning in *Bombyx mori* has been observed to fall to 0.6 suggesting conversion of fat to carbohydrate[13]. Near the end of pupal diapause in *Samia cynthis*, glycerol is converted into glycogen[14]. Infact, considerable amount of carbohydrate is found in many insects and they constitute an important nutrient reserve in preadult stages for adult differentiation[15, 16]. The female adult of *A. mylitta* has significantly greater amount of lipid than male (Table 1). This finding agrees with the contention of other workers[7, 17] who have correlated higher lipid content of adult female with the use of lipid in egg development.

During development, the average weight of larva increases from 0.06 g (I instar) to 28.0 g (V instar)[18]and amount of protein increases from 9.2 mg to 3350 mg, but on dry weight basis, protein content decreases from around 64% to 59% (Table 1). During pupal and adult life, the level is maintained around 59–60%. Evidence[19] is available that in lepidopteran insects there is an extensive synthesis of protein from free amino acids during growth, particularly, at metamorphosis. However, in spite of the radical processes of histolysis and histogenesis in the pupa, there are very small changes in the total amount of protein[6, 9, 20].

Water content of *A. mylitta* shows steady increase during larval life and decrease at pupation. A slight increase is observed in late pupa followed by a significant decrease in the adult (Table 1). These results are fully comparable with observations made on other lepidopteran insects[10, 21].

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Parathyroid gland of *Varanus flavescens* in response to porcine calcitonin administration

(Key words : calcitonin/hypocalcemia/hypophosphatemia/parathyroid/*Varanus flavescens*)

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Abstract

Intraperitoneal (i.p.) injections of porcine calcitonin (0.5 unit/100 g body weight, daily) induces hypocalcemia and hypophosphatemia in *Varanus flavescens*. The treatment activates parathyroid gland.

Introduction

Endocrine regulation of calcium in reptiles deserves special attention because reptiles represent the mainstream of tetrapod evolution having descended from amphibians (the first land vertebrates) and having phyletic affinities with both birds and mammals. Furthermore, they represent the earliest class of vertebrates to achieve complete independence of an aquatic environment[1, 2]. Unfortunately, studies of calcium regulation in reptiles are very limited. Recent studies have shown that the ultimobranchial body (UBB) of reptiles contains calcitonin-producing C cells[3,4] but the role of this hormone (calcitonin) in the reptilian calcium homeostasis has not yet been clearly defined[5]. Reptilian ultimobranchial extracts are hypocalcemic in rat bioassay[6–8] but are ineffective in individuals of their own species[5,6, 9–10]. Since parathyroid hormone is a predominant hypercalcemic principle in reptiles[11–13], any alteration in the serum calcium level should affect its activity. So far, the activity of parathyroid gland of reptiles in response to exogenous calcitonin administration has not been studied. An attempt has, therefore, been made to record the changes undergone by the parathyroid gland, serum calcium and inorganic phosphate levels of *Varanus flavescens* in response to porcine calcitonin administration.

Materials and Methods

Fifty male *Varanus flavescens* Gray (Squamata : Reptilia) (body weight 450–700 gm) were acclimatized under the laboratory conditions in the month of August for a week prior to use. Then, they were randomly divided into two equal groups :

Group A : control animals were injected (i.p.) with 0.1% gelatin (vehicle) at a dosage of 0.1 mg/100g body weight daily.

Group B : experimental specimens were injected (i.p.) with procine calcitonin (Armour Pharmaceutical Company, USA : Lot No. K700–1470, dissolved in 0.6% NaCl containing 0.1% gelatin) at a dosage of 0.5 unit/100 g body weight daily.

Animals from both the groups were not fed during the entire course of investigation. To avoid the effects of circadian rhythm, the injections were given at 10 a.m. and blood samples were always collected at 12 noon throughout the experiment. Blood samples from both the groups were collected under ether anaesthesia directly from the cardiac puncture at 1, 3, 5, 7 and 14 days following onset of the experiment. The analyses of serum calcium and inorganic phosphate were made by Trinder[14], and Fiske and Subbarow[15] methods, respectively.

Parathyroid gland along with the surrounding tissues were extirpated and fixed in aqueous Bouin's solution. After routine processing of dehydration, they were embedded in the paraffin wax. Serial sections were cut at 5 – 6 μ and stained in hematoxylin-eosin. The nuclear size of chief cells were measured with the aid of an ocular micrometer. Each nucleus was measured along its long and short axes and the mean value was calculated. From each group 250 nuclei were randomly measured at every interval (50 nuclei from each specimen). The mean value listed in the Table 1 refers to the means of the mean value per animal.

The differences between serum calcium, inorganic phosphate levels and the nuclear size of both the groups were evaluated for statistical significance using Students' 't' test.

Results

Changes in the serum calcium, inorganic phosphate levels and the nuclear size of parathyroid chief cells in both the groups of *Varanus flavescens* have been summarized in Table 1.

Serum calcium level of the experimental animals records a decline on day 1. The hypocalcemic peak is attained on day 3, and by 7th day, normocalcemia is recorded (Fig. 1). Correspondingly, serum inorganic phosphate also recorded a decline in calcitonin-treated specimens on day 1. The hypophosphatemic peak is recorded on day 3, and by day 7, normophosphatemia is achieved by the experimental animals (Fig. 1).

Varanus flavescens possesses a single pair of parathyroid gland located near the bifurcation of the carotid artery in the cervical region (Fig. 2). The gland is compact organ surrounded by thick connective tissue capsule. The chief cells are often arranged in cords but sometimes follicles with eosinophilic colloid-like material in their lumina are also encountered (Fig. 3).

Parathyroid gland of control *Varanus flavescens* comprises sparsely distributed chief cells with rounded nuclei (Fig. 4). Not much of histological change is seen in the gland of control specimens as well as among the individuals of experimental group till day 1. However, from

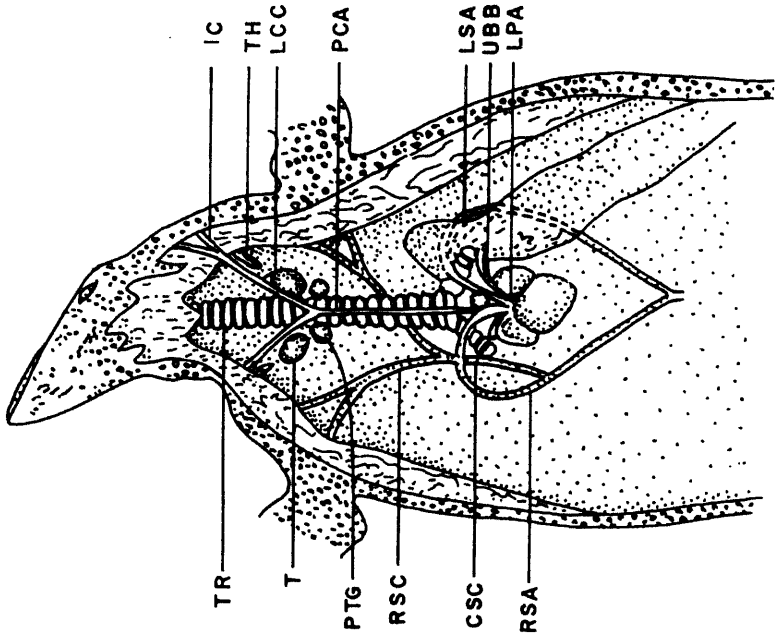

Fig. 2. Location of ultimobranchial body, parathyroid and thyroid in *Varanus flavescens* in relation to arterial supply (CSC, common subclavian artery; IC, internal carotid artery; LCC, left common carotid; LPA, left pulmonary artery; LSA, left systemic arch; PCA, primary carotid artery; PTG, parathyroid gland; RSA, right systemic arch; RSC right subclavian; T, thyroid; TH, thymus; TR, trachea; UBB, ultimobranchial body).

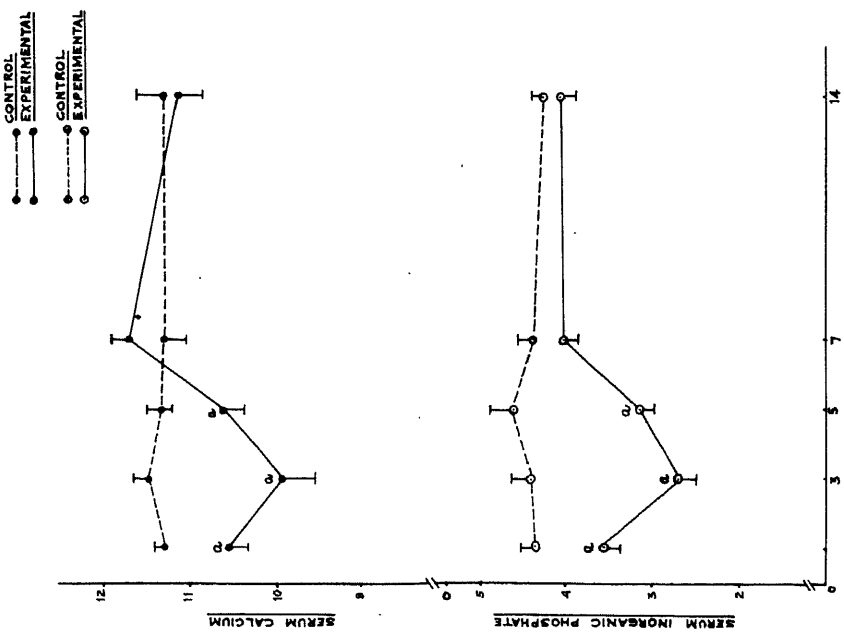

Fig. 1. Serum calcium and inorganic phosphate levels (mg/100 ml) of male *Varanus flavescens* in response to porcine calcitonin administration. Each point represents mean ± S.D. of 5 determinations. a, indicates significant response, $p < 0.001$.

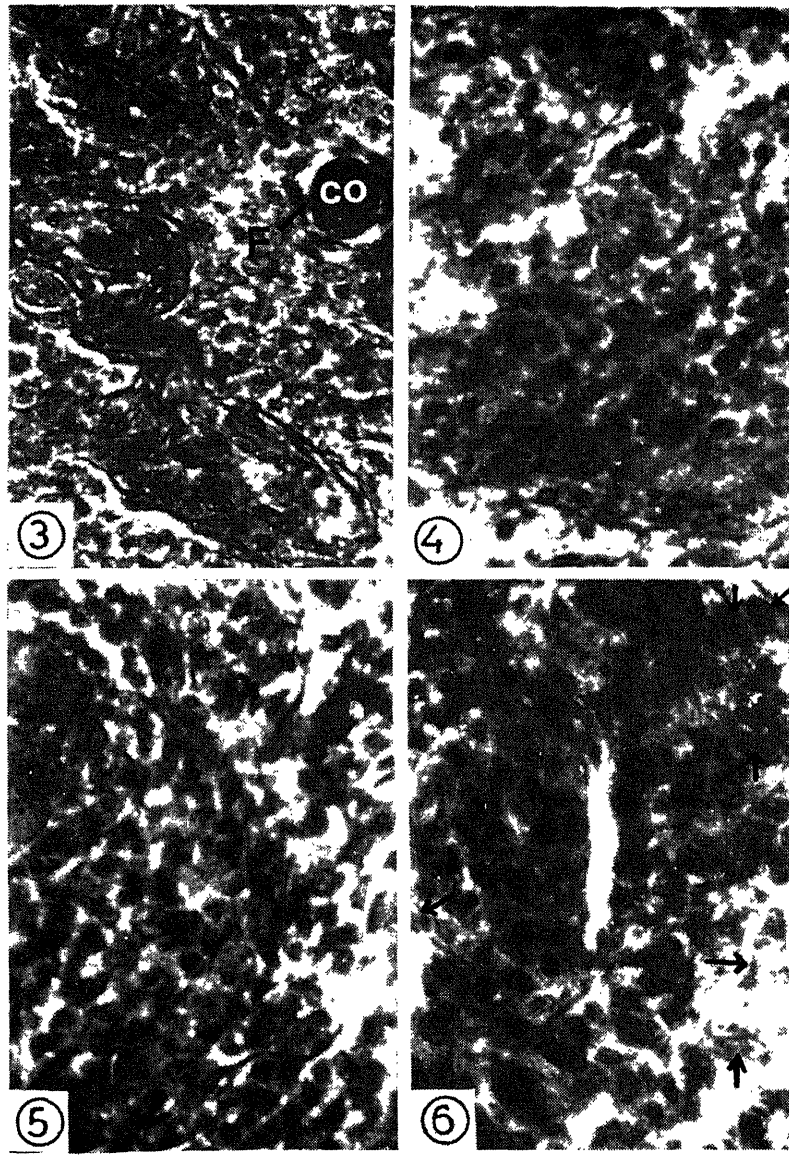

Fig. 3. Parathyroid gland of male *Varanus flavescens* (control) showing the arrangement of chief cells in follicle (F) which contains eosinophilic colloid-like material (CO) in its lumen. Hematoxylin-eosin x 500.

Fig. 4. Parathyroid gland of male *Varanus flavescens* (control) exhibiting sparsely distributed chief cells with rounded nuclei. Hematoxylin-eosin x 580.

Fig. 5. Parathyroid gland of male *Varanus flavescens* after 7 days of calcitonin administration. Mark an increase in the nuclear size and granulation. Hematoxylin-eosin x 580.

Fig. 6. Parathyroid gland of male *Varanus flavescens* after 14 days of calcitonin-treatment depicting hypertrophied chief cells. Few chief cells with elongated nuclei (arrow) are also seen. Hematoxylin-eosin x 580.

Table 1– Effect of porcine calcitonin (pCT) administration on serum calcium, inorganic phosphate levels (mg/100 ml) and nuclear size (μm) of parathyroid chief cells of *Varanus flavescens*.

Day	Serum calcium		Serum inorganic phosphate		Nuclear size	
	Control	Experimental	Control	Experimental	Control	Experimental
1	11.29 ± 0.40	10.55 ± 0.27[a]	4.37 ± 0.19	3.62 ± 0.21[a]	4.53 ± 0.24	4.59 ± 0.22
3	11.49 ± 0.20	9.90 ± 0.50[a]	4.41 ± 0.19	2.69 ± 0.21[a]	4.49 ± 0.20	4.75 ± 0.24[a]
5	11.34 ± 0.12	10.60 ± 0.28[a]	4.46 ± 0.23	3.12 ± 0.26[a]	4.52 ± 0.23	4.98 ± 0.28[a]
7	11.31 ± 0.11	11.70 ± 0.32	4.39 ± 0.14	4.05 ± 0.12	4.49 ± 0.23	5.25 ± 0.25[a]
14	11.34 ± 0.50	11.12 ± 0.20	4.28 ± 0.10	4.06 ± 0.17	4.51 ± 0.22	5.36 ± 0.23[a]

Each value represents mean ± S.D. of 5 specimens.

[a] indicates significant response ($p < 0.001$).

day 3 onwards of calcitonin administration, a progressive increase in the nuclear size and granulation of chief cells have been observed (Fig. 5, Table1). Few chief cells with elongated nuclei are also encountered (Fig. 6).

Discussion

Although ultimobranchial body of reptiles possesses calcitonin producing C cells[3, 4] and calcitonin is present in the reptilian blood[16], the role of calcitonin in calcium homeostasis is uncertain in this group[5]. Dix *et al.*[17], working on *Anolis carolinensis*, have reported that mammalian calcitonin fails to evoke hypocalcemia both in intact as well as in parathyroidectomized lizards. Later, Kiebzack and Minnich[18] have also observed that administration of salmon calcitonin to *Dipsosaurus dorsalis* is ineffective. On the contrary, in the present study porcine calcitonin administration induces hypocalcemia and hypophosphatemia in unfed *Varanus flavescens*. This derives supports from the observations of Kline[19, 20](*Iquana iquana, Sauromelas obesus*) and Srivastav *et al.*[21] *(Natrix piscator)* who have reported that administration of synthetic salmon calcitonin results in significant hypocalcemia.

The return of serum calcium level of *Varanus flavescens* to normal level on day 7 onwards may probably be due to the increased parathyroid hormone secretion as it is evident by the activity of the chief cells which display a progressive increase in the nuclear size among the experimental specimens.

Recently, Laverty and Clark[5] have convincingly suggested a physiological role of calcitonin in the calcium homeostasis of reptiles. The observed hypocalcemia, hypophosphatemia, enhanced activity in the parathyroid gland and degenerative changes in the ultimobranchial body[22] of *Varanus flavescens* in response to calcitonin administration clearly indicate a role of calcitonin in the calcium metabolism of the yellow monitor.

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Effects of some higher plants and synergistic activity of their essential oils against *Sclerotium rolfsii* Sacc., a foot-rot pathogen of barley

(Key words : *Sclerotium rolfsii*/oils/synergistic activity/phytotoxicity/*Citrus medica*/*Eucalyptus globulus*/*Ocimum canum*

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Abstract

During screening of some plant species for toxicity of their volatile components against the mycelial growth of *Sclerotium rolfsii* Sacc. the leaves of *Ocimum canum, Eucalyptus globulus* and *Citrus medica* were found to be strongly toxic. Their volatile active factors were isolated in the form of essential oils which were tested for toxicity individually and in three combinations. The oil combinations were found to be more fungitoxic than the individual oils. The Oc-Eg and Oc-Cm combinations exhibited a broad fungitoxic spectrum while the Eg-Cm combination possessed a narrow range of toxicity. None of the three oil combinations showed phytotoxic behaviour on seed germination, seedling growth and general morphology of *Hordeum vulgare*.

Introduction

A search for antimicrobial substances from higher plants has been initiated recently[1,2]. Some compounds of higher plant origin have proved successful as natural fungitoxicants because of their nonphytotoxicity, systemicity and biodegradable nature[3–5]. Although there are some reports about the fungitoxic properties of the volatile[6–10] essential oils of some higher plants, little attention has been given to their synergistic activity. However, such information is desirable since the fungitoxic potency of most of the fungicides has been reported to be enhanced due to their synergistic activity. This paper deals with the evaluation of the volatile component (essential oils) of the leaves of some higher plants for toxicity against *Sclerotium rolfsii* Sacc., a pathogen responsible for various serious diseases including foot-rot of barley. It also includes studies of synergistic activity between these essential oils.

Materials and Methods

Fresh leaves, 10 g of each taxa, were macerated with 10 ml of sterilized water (1:1 w/v) and filtered through cheese cloth to collect the filterate. The toxicity of the volatiles in the filterate was determined against *Sclerotium rolfsii* Sacc. by the inverted Petri plate techniques of Bocher[11]. The fungitoxicity was calculated following Dikshit *et al.*[12] and recorded in terms of percentage mycelial inhibition.[13].

The volatile antifungal factors from the fresh leaves of the most effective plants viz. *Ocimum canum, Eucalyptus globulus* and *Citrus medica* were extracted by hydrodistillation in Clevenger's apparatus and were finaly collected in the form of essential oils.

The inhibition of the mycelial growth of test fungus at different concentrations of the essential oils was determined by the poisoned food technique of Grover and Moore[14] using malt extract agar medium. In the other set of experiments, three combinations Oc-Eg, Oc-Cm and Eg-Cm from the three oils of *Ocimum canum, Eucalyptus globulus* and *Citrus medica* were prepared by mixing two oils (1 : 1 v/v) of a time and these mixtures were similarly tested at different concentrations against the test fungus. The fungitoxicity spectra of the three oil combinations were also determined following the poisoned food technique.

The phytotoxicity of the oil combinations was studied with respect to seed germination, seedling growth and general morphology of the host plant following the method of Dikhsit *et al.*

All the experimental sets were kept in triplicate and were repeated twice. The findings presented below are based on mean values.

Results and Discussion

Leaves of *Ocimum canum, Eucalyptus globulus* and *Citrus medica* exhibited strong toxicity against the mycelial growth of the test fungus while other plants were effective in different grades (Table 1). Each of the three oils was found to exhibit absolute inhibition of mycelial growth of the fungus at 3000 ppm, 2000 ppm and 1000 ppm (Table 2). However, all the three oil-combinations were toxic even at 500 ppm and 100 ppm thereby showing synergism in their activity (Table 2). The oil combinations Oc-Eg, Oc-Cm and Eg-Cm inhibited growth of the fungi in different grades (Table 3).

Table 1–Fungitoxicity of volatile components in the leaves of some higher plants using *Sclerotium rolfsii* as the test fungus.

Plant species	Percent mycelial inhibition of the test fungus
Acacia arabica	78.5
Emblica officinalis	65.3
Datura stramonium	71.9
Calotropis procera	76.5
Lawsonia alba	83.5
Azadirachta indica	86.3
Eucalyptus globulus	98.5
Solanum xanthocarpum	61.6
Ocimum canum	100.0
Citrus sinensis	91.6
Citrus medica	96.6
Pinus roxberghii	60.8

Table 2–Inhibition of mycelial growth of *Sclerotium rolfsii* by various concentrations of three individual oils and their combinations.

Essential oils and their combinations	Percent mycelial inhibition of S. *rolfsii* at different concentrations (ppm)				
	3000	2000	1000	500	100
Ocimum canum (Oc)	100	100	100	83.6	68.9
Eucalyptus globulus (Eg)	100	100	100	78.5	63.6
Citrus medica (Cm)	100	100	100	72.7	60.8
Oc-Eg	100	100	100	100.0	81.9
Oc-Cm	100	100	100	100.0	76.3
Eg-Cm	100	100	100	100.0	70.5

Table 3–Fungitoxic spectra of essential oil combinations

Name of fungi	Percent mycelial inhibition of test fungus (ppm)					
	Oc-Eg (1 : 1)		Oc-Cm (1 : 1)		Eg-Cm (1 : 1)	
	500	1000	500	1000	500	1000
Aspergillus niger	96.7	100	94.8	100	71.5	85.6
A. terreus	97.8	100	95.9	100	56.3	61.7
A. fumigatus	98.6	100	96.2	100	69.8	75.3
Penicillium rubrum	100.0	100	98.9	100	64.6	69.5
P. citrinum	100.0	100	99.5	100	53.2	58.6
Fursarium oxysporum	99.5	100	97.4	100	62.8	67.7
F. moniliforme	98.4	100	96.3	100	79.6	85.9
Helminthosporium oryzae	100.0	100	100.0	100	80.8	86.6
Curvularia lunata	100.0	100	100.0	100	39.6	42.5
Cephalosporium sp.	90.5	100	87.5	100	31.9	36.4
Trichoderma harzianum	92.9	100	90.8	100	36.6	39.9
T. viride	91.2	100	90.6	100	37.7	41.6
Rhizoctonia solani	100.0	100	100.0	100	70.8	75.5
Microphomina phaseolina	100.0	100	100.0	100	72.2	77.8

Note : The concentrations shown relate to the mixture of essential oils.

The oil combinations showed no adverse effects on seed germination, seedling growth and general morphology of the barely plants (*Hordeum vulgare*), and so appeared to be nonphytotoxic.

Although synergistic activity amongst several synthetic fungicides has been studied by a number of workers[15–17], more information is required in the case of fungitoxic plant products. In ths study, the fungitoxic activity has been found to be enhanced by combining the oils. This contrasts with the findings of Chaurasia and Vyas[18] who observed a decrease of the activity. The oil combinations of Oc-Eg, Oc-Cm and Eg-Cm exhibited a multifold increase in activity as compared with the individual oils and without showing any phytotoxicity.

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Cytophotometric estimation of nuclear DNA in root and shoot meristems of *Cajanus cajan*

(Key words : *Cajanus cajan*/DNA/root/shoot meristems)

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Abstract

In situ estimation of 4C nuclear DNA was carried out in the shoot and root meristems of nine strains of *Cajanus cajan* (L.) Millsp. The nuclear DNA content was estimated through Feulgen cytophotometry. Varietal differences in DNA amount is significant between the strains. Organ to organ variation in DNA content has been noted. The amount of DNA in the shoot meristem is lower than that of the root meristem. The differences in DNA content in the different organs is associated with differential amplification of DNA during differentiation. The amount of DNA in the strains is not correlated with chromosome length but positively correlated with chromosome volume.

Introduction

The leguminous crop, *Cajanus cajan* (L.) Millsp. is widely cutlivated, specially in the northern part of India. A large number of strains have been raised in different agricultural centres in India through selection and other breeding practices. A study of the interrelationships of these different strains through *in situ* nuclear DNA analysis in different organs is desirable.

The amount of DNA in relation to plant evolution has been a debated issue, often complicated due to the presence of very high homogenous sequences[1–6]. Correlation studies have been carried out between mitotic cell cycle, nuclear and cell volume with DNA content[7–10]. Variation in the amount of DNA during organogenesis, attributed to the amplification of DNA during differentiation has also been associated with differences in protein content[11–15].

In view of the importance of the study of nuclear DNA in the root and shoot meristem and the availability of the technique used to execute this programe, the present work in different strains of *Cajanus cajan* was undertaken.

Materials and Methods

Seed of nine strains of *Cajanus cajan* were obtained through the courtesy of different agricultural centres in India and Nepal.

The seeds were germinated at 25–27°C on moist filter paper in petri dishes. For *in situ* DNA estimation, following feulgen cytophotometry a Leitz Wetzlar Aristophot with microspectrophotometer was used. 4C nuclear DNA values were calculated on the basis of optical density from 100 metaphase plates in each of the strains. The relative arbitrary units of

absorbance thus obtained were converted to picogram units with the help of a standard like *Allium cepa*[16].

For statistical analysis of variance (ANOVA), Sokal and Rohlf's[17] method and Duncan's new multiple range test were adopted[18]. The standard correlation coefficient test was also carried out.

Results and Discussion

The quantitation of 4C nuclear DNA by *in situ* technique revealed significant intraspecific differences in both the organs–root and shoot meristem cells of *Cajanus cajan* (Tables 1 and 3). Their somatic chromosome number 2n = 22 is constant in all the nine strains.

Table 1–ANOVA of nuclear DNA of root meristem in nine strains of *Cajanus cajan* (L.) Millsp.

Source	Degree of freedom	Sum of squares	Mean square	F (a/b)
Between strains	8	473.67	59.20 (a)	86.185
Within strains	891	612.535	0.687 (b)	(P < 0.05)

Table 2–Multiple comparision of DNA in root meristem with non-significant blocks of strains of *C. cajan*.

UPAS–120	AL-15	T-21	Khargone-2	C-11	No-148	Nepal	DL-741	Ja-3
9.016	9.029	9.241	9.776	9.974	10.082	10.320	10.923	11.126

Table 3–ANOVA of nuclear DNA of shoot meristem in nine strains of *Cajanus cajan* (L.) Millsp.

Source	Degree of freedom	Sum of squares	Mean square	F (a/b)
Between strains	8	241.78	30.22 (a)	47.97
Within strains	891	558.49	0.63 (b)	(P < 0.05)

Table 4–Multiple comparision of DNA in shoot meristem with non-significant blocks of strains of *Cajanus cajan*.

NO-148	AL-15	UPAS-120	Khargone-2	Nepal	DL-74-1	C-11	T-21	Ja-3
7.795	7.815	8.020	8.315	8.437	8.565	8.656	8.868	9.524

The shoot meristem cells showed a lower value of DNA content than the corresponding root meristem in all the strains. The least amount of nuclear DNA in the shoot meristem may be explained by the fact that it represents the initiation point from which different organs differentiate gradually[14, 15]. The difference in the amount of nuclear DNA between the two organs was significant in all the strains. The increase in the amount of DNA in the root meristem is obviousiy due to lateral amplification of DNA during organogenesis[19-22]. However, this amplification is not identical in all the strains as indicated by the four different sets of non-significant groups of strains adopted following Duncan's multiple range test (Tables 2 and 4). It is quite clear that amplification of DNA is genotype specific which differs from one another depending on the need of the genotype adapted under specific environmental setup. The least DNA amplification is observed in the strains UPAS–120 and the highest in the strain No–148 in the root meristem.

In the meristematic cells of the root, the total length of the chromosomes shows no correlation with nuclear DNA amount. The variation in chromosome length is, therefore, due to differential spiralization and consequent condensation of chromosomes which is directly associated with repression and derepression of gene sequences under genetic control. However, a positive correlation has been noted between total chromosome volume and DNA content (Table 5). Amplification of DNA takes place laterally and not in tandem sequences. Therefore, with lateral increase of DNA during organogenesis there is a positive correlation with chromosome volume in the strains of *Cajanus cajan.*

Table 5–*In situ* nuclear DNA in root and shoot meristems of *Cajanus cajan* and chromosome size

Strains of *Cajanus cajan*	Total length (a)	Total volume (b)	4C DNA of Root (c)	Shoot (d)
UPAS-120	44.38	23.8	9.016	8.020
AL-15	54.90	24.6	9.029	7.815
T-21	48.08	23.3	9.241	8.868
Khargone-2	48.16	25.9	9.776	8.315
C-11	52.66	25.4	9.974	8.656
NO-148	49.04	30.1	10.082	7.795
Nepal	63.48	36.7	10.320	8.437
DL-74-1	51.36	38.7	10.923	8.565
Ja-3	45.58	31.3	11.126	9.524

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Occurrence of vesicular arbuscular mycorrhiza in medicinal plants

(Key words : V-A mycorrhiza/medicinal plants)

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Abstract

Thirty three medicinal plants belonging to twenty different families were surveyed for vesicular-arbuscular mycorrhizal (VAM) colonization. All of them were mycorrhizal. However, the intensity of mycorrhization and rhizoplane/rhizosphere spore formation varied with the plant species and the stages of their growth.

Introduction

The possibility of inoculating crop plants with selected strains of vesicular-arbuscular mycorrhizae (VAM) more effective than that already present in soil has improved the prospects of their field performance. Evidences show that VAM increases plant growth through improved nutrient uptake especially phosphate and water[1, 2]. Screening plant cultivars for degree of VAM root infection gives an indication of plant types more susceptible to colonization with better ability of performance under natural conditions.

Increasing attention is presently being paid to the medicinal plants because of their socio-economic importance. Results of an extensive survey of VAM formation in them was, therefore, undertaken and the findings are reported in the present communication.

Materials and Methods

Different plant species known to have medicinal importance were collected at periodical intervals from different sites at Allahabad. The area included in the survey had been exposed to a temperature range of 7 to 44°C, humidity range of 8 to 95% and received 75 cm annual rain fall during the survey period. Its soil is sandy clay loam, having pH from 6.8 to 7, with organic matter 0.72% and shows a natural population of mainly *Glomus* (VAM) ranging from 3 to 4 spores/g oven dry soil.

Samples containing plant roots and adhering soil were collected and brought to the laboratory under aseptic conditions. The roots were washed repeatedly and cut into small segments of approximately 1 cm each. To record the intesity of VAM formation, 100 segments selected at random were cleared in 10% KOH solution for 1 h at 90°C and stained in .05% Trypan blue in lactophenol for about 1 h. The segments were examined for VAM and the intenstiy of mycorrhiza formation was expressed in terms of per cent segments showing presence of VAM.

Table 1–Intensity of mycorrhization in medicinal plants at different stages of growth (numericals within parenthesis show spore population in terms of spores/g oven dry soil).

Plants	Family	% root segments showing VAM formation			
		Stages of growth			
		Vegetative	Flowering	Fruiting	Average
Elettaria cardamum L.	Zingiberaceae	00.0(10.0)	30.0(2.0)	15.0(2.0)	15.0(5.0)
Amomum subulantum Roxb.	"	37.0(3.0)	45.0(5.0)	33.0(12.0)	37.1(7.0)
Acalypha indica L.	Euphorbiaceae	40.0(20.0)	23.0(9.0)	50.0(1.0)	37.6(10.0)
Riccinus communis L.	"	00.0(2.0)	15.0(5.0)	34.0(8.0)	16.3(5.0)
Hyoscyamus niger L.	Solanaceae	40.0(17.0)	55.0(22.0)	60.0(25.0)	51.6(21.0)
Datura stramonium L.	"	33.7(13.1)	50.0(01.0)	75.0(00.0)	56.9(5.0)
Aloe barbadensis Mill.	Liliaceae	100.0(07.0)	47.0(25.0)	100.0(5.0)	82.3(12.0)
Cannabis sativa L.	Cannabinaceae	15.6(02.0)	53.0(04.0)	66.6(09.0)	45.8(5.0)
Catharanthus roseus L.	Apocynaceae	73.0(79.0)	75.0(13.0)	65.0(80.00	71.0(57.0)
Rauwolfia tetrafolia L.	"	52.0(09.0)	62.5(02.0)	100.0(02.0)	70.8(04.0)
Rauwolfia serpentina L.	"	68.0(05.0)	47.0(06.0)	08.6(02.0)	41.2(04.0)
Rosa alba L.	Rosaceae	47.4(04.0)	19.2(02.0)	10.2(02.0)	25.5(04.0)
Papaver somniferum L.	Papaveraceae	100.0(20.0)	00.0(20.0)	00.0(07.0)	33.3(16.0)
Andrographis paniculata Nees.	Acanthaceae	34.0(05.0)	25.0(02.0)	16.0(01.0)	25.0(03.0)
Adhatoda vesica Nees.	"	73.5(03.0)	25.0(17.0)	40.0(02.0)	46.1(08.0)
Ruellia tuberosa L.	"	46.8(05.0)	80.0(02.0)	44.4(02.0)	57.1(03.0)
Hibiscus sabadariffa L.	Malvaceae	50.0(06.0)	32.4(02.0)	18.7(02.0)	33.7(03.0)
Plumbago zeylanica L.	Plumbaginaceae	65.0(05.0)	24.0(11.0)	14.0(13.0)	34.3(10.0)
Abrus precatorius L.	Papilionaceae	71.0(21.0)	31.4(05.0)	12.0(10.0)	38.1(12.0)
Psoralea corylifolia L.	"	37.5(05.0)	41.0(06.0)	00.0(02.0)	26.1(05.0)
Grotalaria prostrata Rotll.	"	72.0(09.0)	23.0(13.0)	38.8(04.0)	44.6(09.0)
Tephrosia purpurea Pers.	"	40.0(05.0)	15.0(01.0)	04.0(04.0)	18.1(03.0)
Gemnema sylvestire R.	Asclepiadiaceae	67.0(09.0)	37.5(04.0)	10.0(02.0)	38.1(05.0)

Table 1 Contd.

Plants	Family	% root segments showing VAM formation			
		Stages of growth			
		Vegetative	Flowering	Fruiting	Average
Hemidesmus indicus R.	"	49.2(27.0)	27.0(19.0)	63.8(02.0)	49.6(16.0)
Tylophora fasciculata Ham.	"	40.1(08.0)	45.4(05.0)	53.3(04.0)	46.2(06.0)
Mimosa pudica L.	Mimosaceae	40.0(13.0)	26.0(23.0)	48.0(03.0)	38.0(13.0)
Cissus guandrangularis L.	Vitaceae	41.2(03.0)	37.5(06.0)	06.0(02.0)	28.2(04.0)
Curculigo orchioides Gaertn.	Amaryllidaceae	48.4(01.0)	27.0(02.0)	16.6(00.0)	30.6(01.0)
Pandanus tectorius Soland	Pandanaceae	39.0(08.0)	44.1(05.0)	78.5(10.0)	53.8(08.0)
Ocimum basilicum L.	Labiatae	27.7(04.0)	50.0(42.0)	11.0(53.0)	29.5(33.0)
Ocimum sanctum L.	"	25.0(03.0)	65.0(20.0)	00.0(25.0)	30.0(13.0)
Artemesia maritima L.	Amaranthaceae	66.6(02.0)	44.0(02.0)	14.0(01.0)	41.5(02.0)
Kalanchoe pinnata Pers.	Crassulaceae	30.0(06.0)	90.6(05.0)	00.0(02.0)	40.0(04.0)

Minimum difference required for significance (C.D.) at 5% level; Percentage root infection : 3.33; Spore population : 11.92

Root washings were employed for evaluating the population of *Glomus*(VAM) spores in the vicinity of roots. The spores were extracted from the root washings by wet sieving and decanting procedure[3] and counted under a stereoscope. The population of spores was computed and expressed in terms of number of spores/g oven dry soil.

Three replicates were taken in each case and the data were analysed statistically[4].

Results and Discussion

All the plant species surveyed were found to be mycorrhizal. However, the intensity of root infection varied with plant species. Similar variations were also recorded in the magnitude of sporulation of VAM endophyte (Table 1). It is evident from the data that intensity of the mycorrhizal infection as well as sporulation fluctuated with the age of plants. Besides specificity and preference of the VAM endophyte for different hosts[5–7], there are possibly many plant factors that may be attributed to explain the different rate of root colonization by VAM fungi in the present investigation. Based on the stages at which the plant and fungus interact, these factors may be the differences in host fungal recognition, caused by changes in quality and quantity of root exudates, and the VAM fungal attraction towards the growing plant root; susceptibility of plant roots to fungal penetration, and root surface characteristics affecting the number of infection sites. Also the intensity of VAM fungal spread inside the root is a function of its interaction with the anatomical and physiological status of the root. Quantities of substances that are either inhibitory (phytoalexins) or stimulatory (carbohydrates) and the ratio of inhibitory and stimulatory compounds determine the rate of fungal proliferation. The extent of root colonization by VAM fungi is also possibly a plant-heritable trait, that can be compared to that of the plant genes involved in resistance or surceptibility to plant pathogenic fungi[8]. A comparison of the mycorrhizal infection in roots and sporulation in different plant species shows that a direct correlation between the two, in most of the cases, was lacking. In a number of plant species, heavy root infection was coupled with low sporulation or a lower root infection with heavy sporulation. Only in few cases, the magnitude of root infection and sporulation was of a similar order. While root infection is related to vegetative growth of the endophyte, the sporulation is to its multiplication potential. Being divergent phases of life, the specific requirements for optimum expression of the two are expected to be different. This might have been the probable reason for lack of a direct correlation between the order of root infection and spore production by the endophyte.

Formation of VAM in all the medicinal plants surveyed is noteworthy. Heavy root infection and sporulation in a number of cases point out to a possibility of employing VAM as resource for improving the performance of medicinal plants and facilitates practical application in the field through selected strains of endophyte.

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Enhancement of IAA induced rooting by spermine : relationship between rhizogenesis, peroxidase and IAA oxidase in *Phaseolus vulgaris* L. hypocotylar cuttings

(**Key words** : hypocotylar cutting/rhizogenesis/peroxidase/IAA oxidase/sperine/*Phaseolus*)

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Abstract

Exogenous application of spermine slightly promoted rooting of hypocotylar cutting of *Phaseolus vulgaris* L. Cv. White Panchmari, but when applied along with IAA, it had an additive effect of IAA induced rooting. IAA-spermine treated cuttings showed highest peroxidase activity in comparision to control and individual treatments which is correlated to maximum number of roots produced.

Introduction

Although the precise physiological functions of polyamines in plants are not known, evidences suggest that they play important role in cell division, proliferation, differentiation and development[1,2]. Similarly, during growth and development the role of peroxidase has also been demonstrated[3]. The isoenzymes of peroxidase and of IAA-oxidase appear during rooting[4]. A continuous rise in peroxidase activity both during primordium initiation and emergence and rise in IAA-oxidase activity only during emergence phase in auxin treated hypocotyl cuttings of *Phaseolus vulgaris* L. Cv. Kentucky Wonder has been reported[5]. In the present paper the synergistic effects of spermine on IAA-induced rooting are reported.

Materials and Methods

Seeds of *Phaseolus vulgaris* L. Cv. White Panchmari were procured from the Department of Agriculture, Himachal Pradesh, India. Seedlings were raised from uniform and surface sterilised (with 0.1% $HgCl_2$) seeds in petri dishes at 25 ± 2°C under continuous illumination provided by three 20W flourescent tubes (11 Wm^{-2}). Five-day old seedlings excised 30 mm below the cotyledons, were placed in glass vials containing 10 ml of appropriate test solutions. Wherever necessary, the level of solutions was maintained by daily addition of distilled water or appropriate solutions. During treatment, the same incubation conditions prevailed as those for raising the seedlings. Number of roots (including root primordia) were recorded after 72,96 and 120 h of incubation. The cuttings were used for each treatment and experiments were repeated twice.

Enzyme assays : For enzymic studies, four cuttings (basal 20 mm region) were homogenized in 0.1 M sodium phosphate buffer, pH 6.5, and centrifuged at 8,000 g for 20 min. at 0°C. Final volume of the supernatant was raised to 5 ml with buffer and maintained at 0°C. Peroxidase and IAA oxidised activities were determined[6,7] and have been expressed as Δ OD_{420} mg^{-1} protein min^{-1} and µg IAA oxidised mg^{-1} protein min^{-1} respectively. Protein

estimation was accomplished according to Lowry *et al.*[8] Experiments were conducted in three replications. The values presented are arithmatic means together with standard deviations indicated by vertical bars.

Results and Discussion

Spermine and Indole-3-acetic acid (3 x 10^{-5} M each) promoted the formation of root primordia and development when applied individually. However, when applied together, spermine further enhanced the IAA-induced rooting and the total effect seems to be additive (Fig. 1). Changes in the pattern of peroxidase and IAA oxidase at 24, 48 and 72 h after incubation in control and treated cuttings reveal that the activity of both the enzymes was higher in treated cuttings at all the stages than those in control cuttings (Figs. 2, 3). Peroxidase activity was observed to be maximum in spermine plus IAA treated cuttings at 48 and 72 h of incubation whch coincides with maximum number of roots produced. Peroxidase activity increased progressively during both initiation and emergence phases of adventitious root formation in spermine plus IAA treated cuttings. IAA-oxidase showed sharp rise in its activity during early initiation and late emergent phases of rooting.

Endogenous polyamines have been shown to increase the mitotic activity and protein and DNA synthesis in oat leaf protoplasts[9]. In mungbean, IBA-induced rooting was associated with concomitant increase in polyamine levels[10]. It is thus possible that polyamines have a regulatory role, alone or in interaction with auxins, in the early events of adventitious root formation i.e. active cell division and initiation of primordia as proposed earlier also[11, 12]. Polyamines might interact with hormones and have themselves been proposed to be growth substances[13]. Increase in peroxidase activity in differentiating systems has been repeatedly shown earlier. Higher peroxidase activity is also associated with lignification[14, 15]. Higher peroxidase activity in auxin treated *Theobroma cacao* cuttings as compared to control has also been reported[16]. That peroxidase catalysed oxidation products are required for differentiation has been suggested[17, 18].

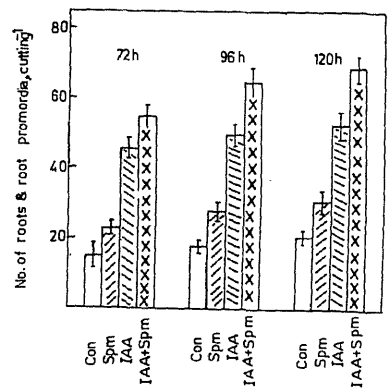

Fig. 1 Adventitious root formation in *Phaseolus vulgaris* L. hypocotylar cuttings as influenced by IAA and spermine (3 x 10^{-5}M each). Vertical bars denote ± S.D.

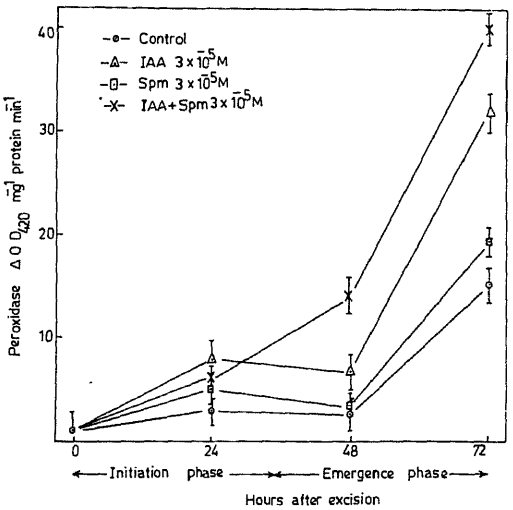

Fig. 2 Peroxidase activity during root initation (determined histologically) and emergence (visual extrusion) accompanying rhizogenesis on hypocotylar cuttings of *Phaseolus vulgaris* L. as affected by spermine and IAA individually and in combination. Vertical bars denote ± S.D.

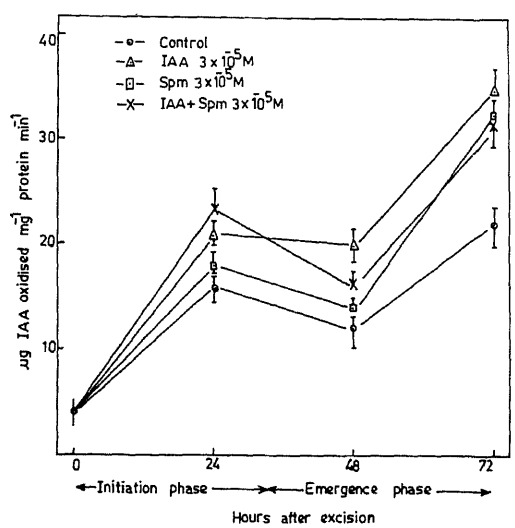

Fig. 3 IAA oxidase activity during root initiation and emergence accompanying rhizogenesis on hypocotylar cuttings of *Phaseolus vulgaris* L. as affected by spermine and IAA individually and in combination. Vertical bars denote ± S.D.

Correlation between polyamines and IAA-oxidase activity has not been shown earlier. Enzymic oxidation of IAA may be essential for positive rooting response, since oxidative products of IAA on conjugation with phenolic co-factors have been implicated in rooting of cuttings[5, 19, 20]. The results suggest that rooting depends on a high metabolic capability for auxin oxidation before the initiation of primordia, and with a relatively lower ability for auxin oxidation during priomordium initiation. Presumably, endogenous auxin levels must rise during primordium initiation. This is consistent with observations that applied auxins may stimulate rooting. On the whole, however, endogenous auxin levels during primordium initiation require further study. Nevertheless, there is no reasonable explanation for the high auxin oxidizing capability that may be required before rooting. In addition, it is unclear whether auxin oxidation products themselves or auxin conjugates, or both, influence rooting directly.

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Electrophoretic variability at five loci in *Zaprionus indianus* populations

(Key words : gel electrophoresis/gene-enzyme systems/allozymes/genic diversity/*Zaprionus indianus*)

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Abstract

Gel electrophoretic analysis was made for three gene-enzyme systems in natural population samples of *Zaprionus indianus* . The data indicate that the three autosomal loci code for codominant and dimeric ACPH; and allelic variants are represented by segregating single band variants. α –GPDH and ADH are under the control of single locus each and homozygous strains depict two banded phenotypes. Data on differential patterns of allelic variability, heterozygosity, Wright's inbreeding coefficient (F) and fit to Hardy-Weinberg expectations at five loci have been discussed with respect to the maintainance of the observed genic diversity in natural populations of *Zaprionus indianus* .

Introduction

Gel electrophoresis constitutes a powerful tool in evolutionary biology, population genetics and systematics[1–2]. The patterns of genetic variability in local, regional and continental species populations of cosmopolitan sibling species pair (*Drosophila melanogaster & D. simulans*) have elucidated some aspects of genetic polymorphism but such information is lacking about other cosmopolitan and colonising species[3–5]. Present investigations have been undertaken to examine the pattern of genic variation occurring at loci coding for three gene-enzyme systems in natural populations of *Zaprionus indianus* .

Material and Methods

The species individuals were bait-trapped from three sites (Chandigarh, Rohtak & Roorkee) in August-Sept., 1987 and were characterised taxonomically and maintained as isofemale lines. Homogenates of single individuals were applied to 12% starch gels, (which accommodate about 15 samples) and run electrophoretically at 250 V and 30 mA at 4°C for 4 hours; α-glycerophosphate-dehydrogenase (α-GPDH) and alcohol dehydrogenase (ADH) isozyme patterns[6]. Genetic control analyses of electrophoretic variation for three gene-enzyme systems were interpreted from the segregation patterns of enzyme electromorphs of parents, F_1 & F_2 progeny of several genetic crosses. The genetic interpretation of banding phenotypes and calculation of genetic number of alleles (n_e), Wright's inbreeding coefficient (F) were followed from standard sources[7–8]. The log-likelihood χ^2 test (G-test) was used to assess whether observed genotypes are in agreement with those expected on the basis of Hardy-Weinberg expectations.

Results

Electrophoretic phenotypes of acid phosphatase, alcohol dehydrogenase and α-glycerophosphate dehydrogenase in single individuals of *Zaprionus indianus* have been

presented in Fig. 1. The gel slice stained for ACPH revealed three zones of activity, each represented by segregating single band variants and three band patterns. However, a single zone each for ADH and α-GPDH is represented by segregating two banded and four banded patterns. Genetic crosses between individuals having triple banded ACPH patterns and those with single band ACPH patterns produced about equal proportions of offsprings with electrophoretic phenotypes like those of the parents (i.e. text crosss ratio of 1: 1). Genetic crosses among individuals having triple banded ACPH patterns revealed 1:2:1 ratio of alternating single banded and triple band patterns; and are thus in agreement with monogenic Mendelian inheritance. Such results were obtained for each of the three ACPH zones and the banding patterns did not vary with the sex. The ACPH phenotypes in *Zaprionus indianus* are thus coded by three autosomal loci. The single band and triple band patterns represent homozygous and heterozygous genotypes respectively and that the ACPH are dimeric in nature. The observed electrophoretic ACPH phenotypes at ACPH-1 to 3 zones are controlled by two, four and two alleles respectively. The genetic crosses involving segregating two banded ADH patterns resulted in four banded phenotypes in F_2 progeny. Similar results were obtained with α-GDPH phenotypes. Thus electrophoretic phenotypes of ADH as well as α-GPDH are under the independent control of a single locus.

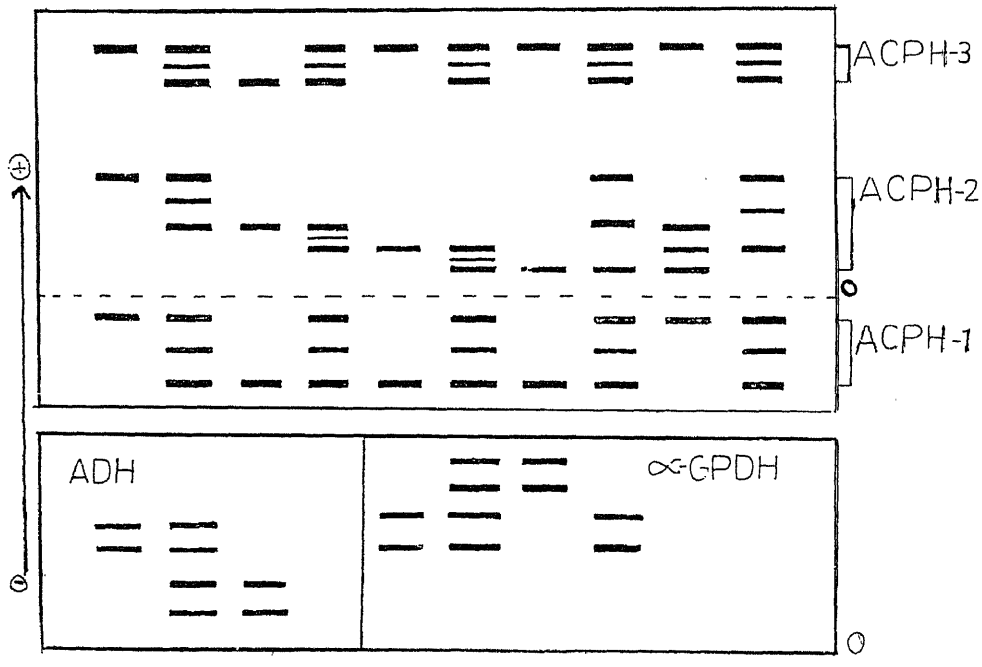

Fig.1. Schematic representation of patterns of electrophoretic phenotypes of acid phosphatases (ACPH), alcohol dehydrogenase (ADH) and α-glycerophosphate dehydrogenase (α-GPDH) in single individuals of *Zaprionus indianus*. Single banded and three banded ACPH phenotypes represent homozygous and heterozygous genotypes repectively. Two banded and four banded ADH/α-GDPH phenotypes represent homozygous and heterozygous genotypes. Arrow indicates the direction of current flow and O represents the origin.

Table 1–Distribution of genotypes, allelic frequencies, heterozygosities, effective number of alleles (n_e), inbreeding coefficient (F) and log likelihood χ^2 test (G-values) for Hardy-Weinberg equilibrium at α–GPDH, ADH and ACPH loci in three populations of *Zaprionus indianus*.

Enzyme Population (N)	Data on genotypes (Obs/Exp) FF	SS	FS	Allele freq. F	S	Heterozygosity Obs/Exp	n_e	F	G-value
α-GPDG									
1.(174)	3/0.85	153.150.50	18.22.65	.07	.93	.10/.13	1.15	0.23	4.324*
2.(150)	2/0.37	137/135.38	11/14.25	.05	.95	.073/.095	1.10	0.23	4.273*
3.(136)	4/1.05	116/113/12	16/21.83	.088	.912	.117/.16	1.19	0.27	6.576*
ADH									
1.(205)	6/11.81	113/118.41	86/74/78	.24	.76	.41/.38	1.57	–0.08	3.91*
2.(198)	9/12.87	106/109.90	83/75.23	.255	.745	.42/.37	1.58	–0.135	2.23 n.s
3.(227)	16/16.65	121/120.96	90/89.40	.27	73	.396/.394	1.65	–.005	0.03 n.s.
ACPH–1									
1.(105)	40/47.14	5/11.43	60/46.43	.67	.33	.57/.44	1.78	–0.29	9.58*
2.(100)	36/40.96	8/12.96	56/46.08	.64	.36	.56/.46	1.85	–0.217	4.69*
3.(140)	60/65.93	8/13.80	72/60.35	.686	.314	.51/.43	1.75	–0.186	5.55*
ACPH–3									
1.(102)	68/65.93	6/3.92	28/32.15	0.804	.196	.274/.316	1.46	0.133	1.57 n.s.
2.(126)	90/88.91	4/3.22	32/33.87	.84	.16	.254/.269	1.37	0.056	0.29 n.s.
3. (104)	64/64.91	4/4.58	36/34.51	.79	.21	.346/.332	1.49	–0.042	0.43 n.s.

N = sample size; * significant at 5% level; n.s. = non significant. Populations : 1 = Chandigarh; 2 = Rohtak; 3 = Roorkee.

Table 2 Distribution of cathodal (ACPH–1) & anodal (ACPH–3) phenotypes, allelic frequencies, heterozygosities and G-values for the log-likelihood ratio test for Hardy-Weinberg expectations in three population samples of *Zaprionus indianus*.

Genetic variability at acid phosphatase (ACPH) loci in three populations

Genotypes Obs./Exp.	ACPH — 1 locus			ACPH — 3 locus		
	Chandigarh	Rohtak	Roorkee	Chandigarh	Rohtak	Roorkee
FF	40/47.14	36/40.96	60/65.88	68/65.93	90/88.91	64/64.91
SS	5/11.43	8/12.96	8/13.80	6/3.92	4/3.22	4/4.58
FS	60/46.43	56/46.08	72/60.32	28/32.15	32/33.87	36/34.51
Sample size (N)	105	100	140	102	126	104
Allele freq.						
F	0.67	0.64	0.686	0.804	0.84	0.79
S	0.33	0.36	0.314	0.196	0.16	0.21
Heterozygosity						
Obs.	0.57	0.56	0.51	0.274	0.254	0.346
Exp.	0.44	0.46	0.43	0.316	0.269	0.332
Wright's inbreeding coefficient (F)	–0.29	–0.217	–0.186	0.133	0.056	–0.042
G-value	9.578*	4.688*	5.553*	1.574 (n.s.)	0.29 (n.s.)	0.428 (n.s.)

* Significant at 5% level; n.s. = non-significant

The data on distribution of ACPH genotypes, allelic frequencies, observed and expected heterozygosity, Wright's inbreeding coefficient (F) and log-likelihood χ^2 test for fit to Hardy-Weinberg expectations at the five loci are given in Tables 1 and 2. The range of heterozygosities observed at 3 ACPH loci correlated well with the incidence of number as well as allelic frequencies distribution patterns. The lower F values indicate that the population samples are randomly mating. Data in Table 1 and 2 reveal that the populations deviate from Hardy-Weinberg equilibrium at ACPH-1 and ACPH-2 loci and there is significant excess of heterozygotes. However, all the three populations depict homogeneity in their genic diversity patterns at ACPH loci.

Electrophoretic analysis at α-GDPH locus in *Zaprionus indianus* has revealed occurrence of one most common allele (0.912 to 0.95) and rare allele (0.05 to 0.08). The population samples have shown lower heterozygosity values and deviate from Hardy-Wienberg expectations (Table 1). The *Zaprionus indianus* populations are characterised by occurrence of two common ADH alleles, high heterozygosity, lack of deviation from random mating as well as from Hardy-Weinberg expectations. However, the patterns of genic variation are nearly the same in three populations.

Discussion

The occurrence of two banded electrophoretic phenotypes of ADH and α-GPDH in homozygous strains of *Zaprionus indianus* significantly differs from the single banded ACPH phenotypes which represent allozyme (allelic isozymes). The present observations on ADH and α-GPDH concur with earlier reports in *Drosophila melanogaster* that in NAD requiring dehydrogenases, more than one electromorph (conformational isozymes) may arise due to post translational differential binding of coenzyme NAD[9]. Genetic tests on electrophoretic phenotypes in Z. *indianus* also support this view.

A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95 and accordingly all the five loci analysed in *Zaprionus indianus* are polymorphic. However, ACPH and ADH loci are significantly polymorphic (with respect to number of alleles, allelic frequencies, and heterozygosity) as compared to α-GPDH locus. The observed low level of genic polymorphism at α-GPDH locus concurs with the functional constraint hypothesis[10]. The mechanism of maintenance of genetic polymorphisms are currently being argued on the basis of Selctionists and Neutralists hypotheses[11-12]. According to Neutralists, patterns of random genetic differences between populations are expected while occurrence of uniformity has been taken as evidence of operation of some type of natural selection at the loci. The present studies have revealed almost homogeneity patterns of genic variation at five loci in the three populations of *Zaprionus indianus* and this could be interpreted due to the action of some type of natural selection. Furthermore, observed deviations from Hardy-Weinberg expectations also support such a contention.

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Conservation and production studies of Gomati riparian ecosystem

(Key words : silt load/riparian/primary productivity/conservation value/water run-off)

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Abstract

The paper deals with the changes in the silt load of the River Gomati at Jaunpur (India), the significance of riparian herbaceous plant community in soil and water conservation, and its productive value. The maximum silt load carried by the river during rainy seasons was 132863 ton day^{-1} in thousand hectare metre in September in the midstream. The peak values were comparatively low, i.e. 76551 and 76644 ton day^{-1} in the thousand hectare metre in August on the two sides, right and left of the river respectively. The silt load was much lower during summer and winter seasons. In order to assess experimentally the quantitative roles of plants in binding the soil and retarding water run off and soil erosion six herbaceous species dominant on the river banks were selected. The soil conservation values of selected grasses and sedge were found to range from 87.6 to 95.4%, and of dicotyledon weeds from 52.8 to 93.8%. The water conservation values ranged from 65 to 74% for grasses and sedge, and 27 to 72% for dicotyledon weeds. The net productivity of abandoned land and adjacent cultivated riparian agroecosystem of plant community on curved course (convex side) of the river bank in the three zones—(upper, middle and lower along topographic gradient) showed wide variation.

Introduction

River banks are extemely fragile and delicately balanced ecosystems. These habitats experience frequent erosion, flooding, silting and variety of biotic influences like grazing, scraping, agricultural operation, bathing and washing of clothes, sewage discharge, burning of dead bodies, religious and cultural congregations, etc. In these habitats, due to continuous silting the soils are being maintained in a pedogenetically young condition[1]. Ambasht[2] has advocated the conservation of soil against rain and runoff forces by planting strips of perennial grasses between the agricultural fields at regular intervals, to provide effective check against soil movement without affecting the crops. Kauffman *et al.*[3] reported livestock impacts on riparian plant community, composition, structure and productivity. Recently, the riparian ecosystem management has been taken up to control nonpoint pollution[4].

This paper mainly deals with silt load in the river water, efficiency of herbaceous vegetation in conserving soild and water and net primary productivity of the riparian ecosystem.

Materials and Methods

Study sites and climate : The present study has been carried out along the bank (convex side) of River Gomati at Rouza Ghat of town Jaunpur (25°45'N latitude and 82°43'E longitude), i.e.

the point where the river enters the city. Two sites situated on Gomati River banks were selected (i) abandoned land (50 x 200 m), (ii) cropland or riparian agroecosystem (75 x 125 m). Both sites located on the same side having almost equal degree of slope. On site II mixed crop of wheat and mustard was cultivated during winter season only, as it remains inundated during rainy season and it is extremely dry during summer season. After the crop is harvested, site II is left fallow during rest of the year. Each site was divided into three zones — upper, middle and lower— according to the transect from the top upland to lower river water margin on the bank. The year is divisible into three main seasons *viz.*, rainy (July to October), winter (November to February), and summer (March to June). The monthly variation in mean maximum and minimum temperature, total rainfall, number of rainy days, annual rainfall and level of inundation from the lowest level of river water margin of the study sites are shown in Fig. 1.

(i) *Silt load.* : The water sampling was done at three points (on left side, middle and right side) across the river. The positions of the points were adjusted with the help of marked wire along the river at every sampling time with the changes in volume due to rise and fall in water levels. Silt sampler was used to collect water sampler (one litre) at the depth point representing 60% of the total depth of water by marked wooden rod. The sediments were oven-dried and weighed.

The water velocity was measured by a current meter. The depth was taken by a marked wooden rod to find out the area of the river at three different sections (left side, middle and right side) of river. Discharge, runoff and silt load are calculated by the formula given below[5] :

Discharge (Q) = A x V

where A = Area of that section in m^2

V = Velocity of river water ($m\ s^{-1}$)

Runoff = Q x 0.00864

(in thousand hectare metres)

Silt load = Run off x g^{-1} x 10^4 ton day^{-1}

(in thousand hectare metres).

(ii) *Soil and water conservations* : The soil and water conservation values of six common plant species which grow luxuriantly during rainy season were determined. The selected species are *Cynodon dactylon* (L) Pers., *Phyla nodiflora* (L.) Greene, *Cyperus rotundus* L., *Croton bonplandianum* Baill., *Crotalaria medicaginea* Lamk. and *Digitaria adscendens* (H.B. & K) Hennr.

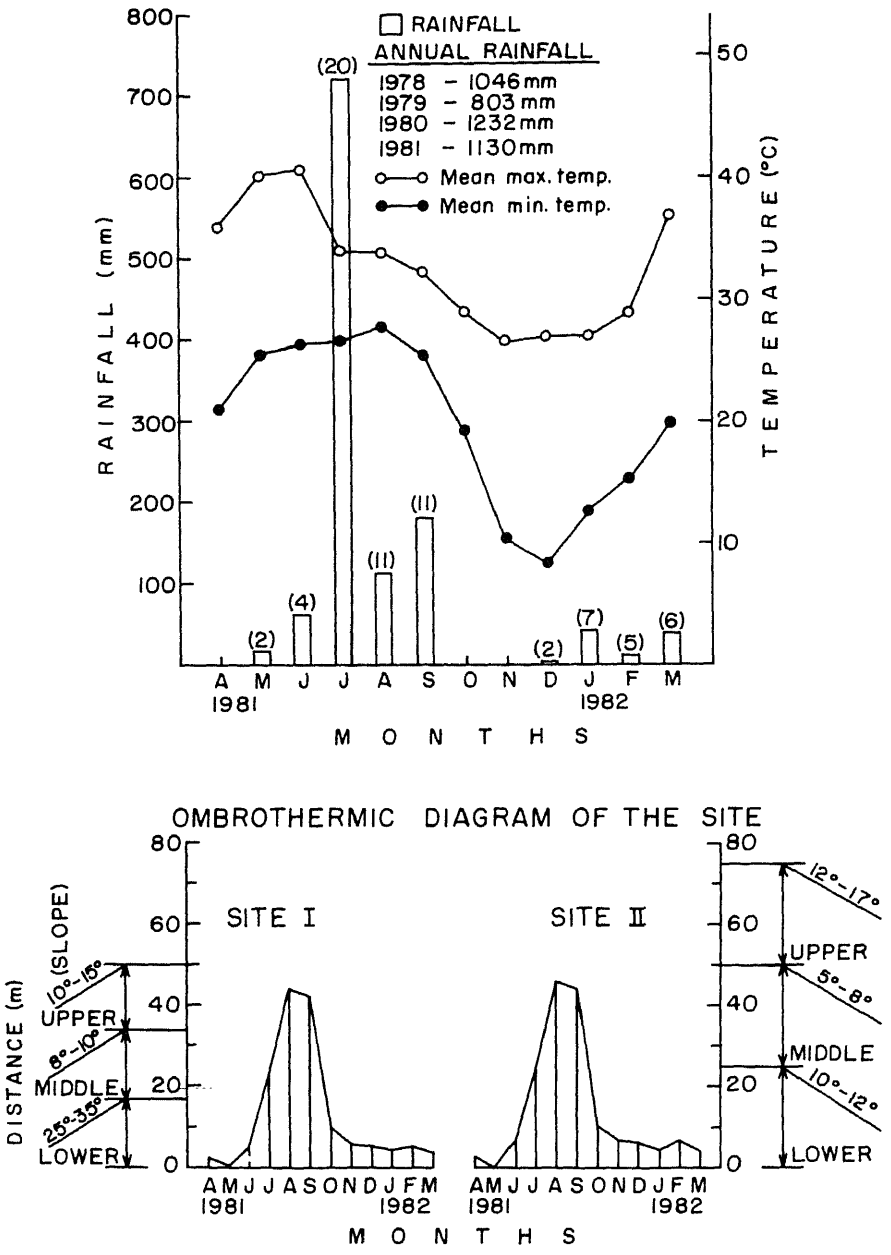

Fig. 1 Degree of slope and level of inundation from minimum water margin.

The propagules of the above species were collected from the river bank and transplanted on plots with 13°slope in the Botanical Garden of Banaras Hindu University, Varanasi. Seven plots (lm x 3m size) were prepared of which three plots were in a east-west direction facing north, and four plots in an east-west direction facing south. One plot was left as such without growing any plant species, whereas each of the remaining six plots were planted with one of six plant species mentioned above. The species were planted at unform density, but their aerial cover increased differently due to difference in their growth pattern. At the base of the slopes, cemented tanks were prepared to catch the runoff water and eroded soil from each plot. The foliage growth was sufficient to cover the ground completely after about 8–10 weeks. Except for careful weeding at an early stage the plant species that grew in the plots in small numbers were not removed to avoid the soil disturbance. Artificial spraying was done for 30 min. using a multipore nozzle with 2 mm diameter perforations from 1 m height at a constant speed of 16 l/min. The showering was done at 15-day intervals for three consecutive fortnights. The runoff water and eroded soil from individual experimental plots including the bare plot were collected. Water sampling was done immediately to avoid any loss. Soil samples were collected and oven-dried at 105°C for 36 hours. Conservation value (CV) of each of the six species was calculated using the following formula given by Ambasht[2] :

$$CV = 100 - \left(\frac{S_{wp}}{S_{wo}} \times 100 \right)$$

where, CV = Soil conservation value,

S_{wp} = Weight of soil washed from vegetated plots,

S_{wo} = Weight of soil washed from the bare plot under identical erosional stresses.

The formula used for soil conservation value was also used for computing the water conservation value of herbaceous plants.The S_{wp} and S_{wo} are replaced by W_{wp} and W_{wo} to denote respectively, weights of water lost from planted and open (bare) plots, and CV refers to water conservation instead of soil conservation[6].

(iii) *Net primary productivity* : Primary productivity studies of the two sites (I & II) bearing weeds and crops were done for the period of one year (April, 1981 to March, 1982). Standing plant dry matter was estimated[7] by "short term harvest method", in which variation is estimated at short intervals. Economic yield of agricultural crop was calculated after separating seeds from husks. The difference of biomass of two consecutive months was taken as net production during that period. Productivity has been calculated on dry weight basis and expressed in terms of g m^{-2} day^{-1}.

Table 1–Approximate silt load carried by the river

Month	Right side				Middle				Left side			
	Discharge Q M^3 Sec^{-1}	Run-off in thsousand hectare metres	Sediment $g\ l^{-1}$	Silt load ton day1 in thousand ha m	Discharge Q M^3 Sec^{-1}	Run-off in thousand hectare metres	Sediment $g\ l^{-1}$	Silt load ton day^1 in thousand ha m	Discharge Q M^3 Sec^{-1}	Run-off in thousand hectare metres	Sediment $g\ l^{-1}$	Silt load ton day1 in thousand ha m
1981												
Apr.	24.950	0.216	0.073	157	90.621	0.783	0.077	602	107.160	0.926	0.666	611
May	18.900	0.163	0.043	70	72.900	0.630	0.056	352	100.800	0.871	0.065	566
June	25.921	0.224	0.510	1142	216.000	1.866	0.366	6829	187.200	1.617	0.299	4834
July	40.920	0.354	1.090	3858	157.081	1.357	1.186	16094	205.921	1.779	0.805	14320
Aug.	680.001	5.875	1.303	76551	1030.000	8.899	1.459	129836	576.000	4.977	1.540	76645
Sept.	595.000	5.141	1.148	59018	1208.001	10.437	1.273	132863	640.004	5.530	1.257	69512
Oct.	62.500	0.540	1.141	6161	342.002	2.955	1.141	33716	302.400	2.613	1.138	29735
Nov.	30.001	0.259	0.600	1554	189.000	1.633	0.945	15431	244.800	2.115	1.017	21509
Dec.	48.000	0.415	0.788	3270	220.503	1.905	0.816	15544	336.600	2.908	0.906	26346
1982												
Jan.	13.282	0.115	0.194	223	46.801	0.404	0.225	909	241.921	2.090	0.351	7335
Feb.	14.040	0.121	0.195	235	126.752	1.095	0.176	1927	213.900	1.848	0.124	2291
Mar.	9.451	0.082	0.078	63	70.090	0.606	0.095	575	239.902	2.073	0.040	829

due to heavy input of rain water washing down soils from catchment areas. At this particular location the erosional forces on the right (convex side) and left (concave side) sides are different due to different forces of water current and the degree of slope.

(ii) *Soil and water conservation* : On the bare plots soil loss was 7.8 kg, infiltration 18.33% and water runoff 81.67%. The quantities of soil eroded from vegetated plots were 0.36 kg from *C. dactylon*, 0.48 kg from *P. nodiflora*, 0.69 from *C. rotundus*, 0.97 kg from *D. adscendens*, 2.50 kg from *C. bonplandianum* and 3.68 kg from *C. medicaginea* plots. The infiltration rate was much higer and run-off was lower in the case of vegetated plots compared to the bare plot (Table 2). The soil conservation values of *C. dactylon*, *P. nodiflora*, *C. rotundus* and *D. adscendens* were 95.4%, 93.8%, 91.1% and 87.6% respectively. The soil conservation values of other two species viz; *C. bonplandianum* and *C. medicaginea* were only 68.0% and 52.8% respectively. The highest water conservation value of 74.23% was obtained in *C. dactylon*, while *P. nodiflora* was the next most efficient species having 72.95% water conservation value. *C. medicaginea* showed progressively lesser values. The high soil conservation value species also showed to a high water conserving efficiency (Table 2).

The soil of the bare plot showed degradation in its properties, while the one with plant cover improves itself in many ways. The plant cover protects the soil against erosion. The plant species mentioned above have high soil and water conserving efficiency and they also retard surface run-off.

Table 2–Variation in water and soil runoff quantities and conservation values.

Plots	Water run-off (kg)	Water run-off (%)	CV for water (%)	Soil loss (%)	CV for soil (%)
Plot 1 *Cynodon dactylon*	101	21.04	74.23	0.36	95.4
Plot 2 *Phyla nodiflora*	106	22.08	72.95	0.48	93.8
Plot 3 *Cyperus rotundus*	114	23.75	70.91	0.69	91.1
Plot 4 *Digitaria adscendens*	136.5	28.44	65.17	0.97	87.6
Plot 5 *Croton bonplandianum*	205	42.71	47.70	2.50	68.00
Plot 6 *Crotalaria medicaginea*	285	59.38	27.29	3.68	52.80
Plot 7 *Bare*	392	81.67	—	7.80	—

CV = Conservation Value

Frequent showerings on plant covered and bare plots depleted the soil and nutrient quantities differently. From bare plot the losses were much more. The mechanical quality of soil eroded from the plots also differed; the one from bare plot had more of clay and silt than from vegetated plots. This is because the beating drops of showered water selectively separate the clay fraction and bring it on the top of the surface soil and it gets eroded more than the lower layer of silt and lowest of sand. Depending upon the nature of vegetal cover, different species prevented the beating effects of water drops to different levels. *C. dactylon, P. nodiflora* and *D. adscendens* with luxuriant foliage cover were most efficient in the above respect.

C. dactylon, P. nodiflora, C. rotundus and *D. adscendens* had high soil conservation values (Table 2) as they formed a thick cushion allowing greater infiltration. The other two species, *C. bonplandianum* and *C. medicaginea,* had moderate soil conservation values. The annual and perennial herbs and shrubs in flood-prone river banks play a significant role in soil and water conservation. River banks need protection from excessive biotic stresses such as over-grazing, scraping and removal of *C. bonplandianum* and *C. medicaginea* for fuel.

(iii) *Net primary productivity* : Primary productivity of plant community is largely influenced by the amount and distribution of rainfall. Community production rate was higher in the rainy season than in summer season. The main factors responsible for the maximum primary productivity are the high moisture, bright light and higer chlorophyll content. During summer season the fresh growth was negligible as compared to the loss in the form of litter that already produced the negative trend of net productivity. The first monsoon shower in the middle of June accelerated the rate of production in the study stands. The peak productivity value of the community was in the lower zone followed by middle and upper zones as the growth proceeded (June). Later flooding created a difference in the periodicity of peak values of the community in the three zones.

At site I, the peak rainy season production rate was in July in the middle zone (5.95 g m^{-2} day^{-1}). After the recession of flood-water the trend of net productivity value of the community was negative in the upper zone in October. On recession of flood-water from the middle and lower zones, the winter annuals showed fresh growth (with staggered germination). The peak production value in the middle and lower zones of site I was in January, whereas in the upper zone peak winter season value was in February (Table 3).

The trend of net primary productivity during fallow period of site II was almost similar to site I, during summer season. The first monsoon shower in the middle of June has accelerated the rate of production in the study stand. The peak productivity value of the community was in the lower zone (4.06 g m^{-2} day^{-1}) followed by middle zone (4.01 g m^{-2} day^{-1}) and lower zone (2.14 g m^{-2} day^{-1}) as the growth proceeded (June). After this the lower zone got flooded while the peak production to values in the middle zone (4.71 g m^{-2} day^{-1}) became maximum followed by upper zone (4.48 g m^{-2} day^{-1}). However, after the recession of flood water in October the trend of net productivity of the community became negative in the upper zone (Table 3).

The values of net primary productivity of site II for both the crops and weeds have shown increasing trend with the advance of age upto 105 days, and then it declined. The peak net

Table 3–Monthly variation in net productivity (g m^{-2} day^{-1}) of total community at site I and during fallow period of site II given in parentheses in three different zones.

Zones	Months											
	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
	1981								1982			
	–0.88	1.84	5.44	2.79	0.87	–2.05	–4.04	1.80	1.82	2.50	–2.59	–3.67
Upper	(–1.10)	(2.14)	(4.48)	2.26	(1.03)	(–2.79)		WINTER CROP*			(–6.50)**	(–0.82)
	–0.89	2.45	5.95	INUNDATED		2.49	1.89	1.75	3.43	0.93	–1.86	–3.33
Middle	(–0.79)	(4.01)	(4.71)	INUNDATED	INUNDATED	2.25		WINTER CROP*			(–9.34)**	_0.89
	–0.66	2.87		INUNDATED		2.26	2.39	2.01	2.95	1.08	–1.43	–3.13
Lower	(–1.34)	(4.06		INUNDATED		2.13		WINTER CROP*			(–8.89)**	(–0.59)

* November to first fortnight of March shows winter crop period

** Values have been calculated by subtracting weeds of crop from weeds of fallow period.

Table 4–Net primary productivity (g m^{-2} day^{-1}) of crops, weeds and total community at site II during winter crop period, in three zones and at different ages.

Zones	Plant	Plant age (days) 15	30	45	60	75	90	105	120
Upper	crops (wheat & mustard)	0.47	0.68	2.86	4.53	7.98	15.88	30.13	9.93
	weeds	0.11	0.39	0.70	1.40	2.09	3.67	4.71	–1.06
	Total for crops and weeds	0.58	1.07	3.56	5.93	10.07	19.55	34.85	8.87
Middle	crops (wheat & mustard)	0.68	1.19	3.04	5.32	9.60	19.50	36.33	12.32
	weeds	0.82	1.57	1.80	2.02	2.37	3.21	3.99	–0.26
	Total for crops and weeds	1.50	2.76	4.84	7.34	11.97	22.71	40.32	12.08
Lower	crops (wheat & mustard)	1.21	2.37	5.42	8.83	16.63	23.13	46.69	16.84
	weeds	1.30	1.71	2.43	2.59	3.71	4.18	1.53	–2.09
	Total for crops and weeds	2.51	4.08	7.85	11.42	20.35	27.31	48.21	14.75

Table 5–Net annual production g m^{-2} yr^{-1}) of total community at site I, total net production (g m^{-2} per eight months) at site II during fallow period and total net production (g m^{-2} per four months) for total crops and weeds at site II during winter crop period, in three zones.

Zones	Site I *(abandoned land)*	Site II *(fallow period)*	*(winter crop period)*
Upper	457.75	297.37	1266.99
Middle	566.65	329.32	1552.61
Lower	406.47	185.63	2047.26

primary community productivity during the crop period of site II was 31.82, 40.32 and 48.21 g m^{-2} day^{-1} between 90 to 105 days age in the upper, middle and lower zones respectively (Table 4). The grain yield in wheat and mustard crop was quite different in the three zones. The peak yield of wheat grain was 2.64 ton ha^{-1} (upper zone), 2.68 ton ha^{-1} (middle zone) and 2.95 ton ha^{-1} (lower zone) per cropping season. The average tiller density for all the zones was 109.44 tillers m^{-2}. Mustard grain yield was 0.26 ton ha^{-1} (upper zone), 0.44 ton ha^{-1} (middle zone) and 1.96 ton ha^{-1} (lower zone) per cropping season with the average plant density of 5.38 plants m^{-2} for all the three zones. It clearly indicates that the lower zone is more productive than other two zones[8, 9].

The highest annual net production of the community at site I was 566.65 g m^{-2} yr^{-1} in the middle zone followed by 457.75 and 406.47 g m^{-2} yr^{-1} in the upper and middle zones (Table 5). The mean value 476.96 g m^{-2} yr^{-1} of net annual production of site I is higher to 228.87 g m^{-2} yr^{-1} at Yamuna river bank at Agra[10]. It is clear that site I is more productive than Yamuna River bank community. In contrast the total net production for entire community, in the fallow period of site II was highest in the middle zone (329.32 g m^{-2} per eight months) followed by the upper zone and least in the lower zone. It may be mainly due to the impact of longer period of inundation (July to September, 1981) in the lower zone of site II, during fallow period. The peak values of net primary productivity of total community of site II, during four months of cropping period were in lower zone (2047.26 g m^{-2}), followed by middle zone (1552.61 g m^{-2}) and upper zone 1266.99 g m^{-2}). More production during winter crop period on lower zone is mainly due to better soil texture, nutrients and moisture[11]. It is clear that convex side of the river bank which receives rainy season recharging on the lower slopes can be used for agricultural practices with certain precautions and protections.

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Genetic inheritance of powdery mildew resistance and its breakage in peas

(Key words : powdery mildew/simple recessive/inheritance)

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Abstract

A breeding programme to incorporate powdery mildew (*Erysiphe polygoni* D.C.) resistance in commercial field & garden peas (*Pisum sativum* Linn.var *arvense & hortense*) and its genetic inheritance was taken up. The powdery mildew resistant donors comprised of T 10, 6588 and 6587; while the commercial susceptible varieties were T 163, Kinnauri, Local batri yellow, Local batri brown, JM 1, JM 2, JM 4, EC 33866, 46 C, R 98 B, Afila and R 710. The F_1 generation was highly susceptible while the F_2 progenies indicated 3 susceptible : 1 resistant ratio meaning thereby that the disease resistance was inherited as a simple recessive gene. Many of these resistant lines reverted back to susceptible conditions right from F_3 to F_{13} generations (er er to Er Er).

Introduction

The pea is highly affected by powdery mildew which is one of the main causes of drastic reduction in production. The screening of pea genetic stock against powdery mildew has been reported earlier by Narsinghani[1]. The best available genotypes were hybridized during 1974–75. The breeding behaviour of powdery mildew resistance in peas proved to be inherited as a simple recessive. However, the reversion back to dominant condition of the gene er er resulting in breakage of resistance could not be explained. This reversion is continuing till today after F_{12} to F_{13} generations.

Materials and Methods

A set of 21 crosses of peas was attempted earlier between powdery mildew resistant donors T 10, 6588 and 6587 with T 163, Kinnauri, Local Batri yellow, Local Batri brown, JM 1, JM 2, JM 4, EC 33866, 46 C, R 98 B Afila and R 710. The F_1 generation was planted in single row plots of 5 m length placed 1 m apart. The F_2 generation obtained after selfing of F1 was planted in large plots and the generations were further advanced to F_3–F_{13}.

The observations on powdery mildew resistance was recorded on a 0–5 scale[2] from F_1 to F_{13} generations as suggested by Munjal *et al.*[2].

The data was analysed statistically by applying Chi-square test and significance of fitness of the genetic ratio was calculated at 5% and 1% probability level.

Results and Discussion

The inheritance of powdery mildew disease was studied earlier in the year 1976-77 and 1977-78. The F_1 generation was invariably highly susceptible to powdery mildew attack, indicating that the dominant genes were responsible for susceptibility of the desease. In F_2 generation, the chi-square test gave a significant ratio of 3 susceptible to 1 resistant. This ratio has been reconfirmed in subsequent years for various combinations of parents. The genes responsible for susceptibility are designated as Er Er and the resistance against powdery mildew in peas in governed by er er.

The F_2 generation was further advanced from F_3 to F_{13} generations. The process of genetic stabilization of the resistance against powdery mildew indicated the breakage of resistance, dilution of resistance as well as reversion back to susceptibility right from F_3 onwards. The reversion back from resistant condition to suscetibility has been seen up to 13 generations. Theoretically, the recessive gene er er should not revert back to the dominant Er Er. The detailed study of this reversion back was carried out during F_6, F_7 and F_8 generations. (Table 1).

Table 1–Reversion of powdery mildew resistance in advancing generations of pea crosses.

	Accession number of progenies	Powdery mildew scale		
		(F 6)	(F 7)	(F 8)
JP	3, 4, 6, 9–4, 15, 21, 22, 24, 39, 41, 92A, 92B, 132, 133, 138, 141, 169, 175, 179, 198, 204, 227, 230, 231, 254, 272 293, 298, 501, 517, 519, 522, 523, 527, 528, 530, 532, 534, 542, 560, 570, 571, 577, 578, 585, 588, 753, 763, 861, 868, 885, 888	0	0	0
JP	16, 107, 108, 124, 248	0	0	1
JP	9, 50A, 50B, 134, 189, 520, 521, 538, 545, 559, 569, 746, 747 748, 779, 854, 884, 886	0	1	1
JP	130, 573, 574, 576, 580, 766, 781, 787, 788, 818, 827, 837, 845	1	1	1
JP	110, 111, 157, 163, 180, 200, 829	0	0	2
JP	98, 187, 297, 770	0	1	2
JP	91, 832, 833	1	1	2
JP	2, 197, 843	0	2	2
JP	191, 849	1	2	2
JP	139,167, 582, 728, 739, 760, 840, 862	2	2	2
JP	114	0	1	3
JP	598	0	2	3
JP	123	1	2	3
JP	129	2	2	3
JP	261, 506, 516, 881	0	1	4
JP	31, 37, 193, 220, 221, 250, 536, 604, 616, 784	0	4	4
JP	914, 937	1	1	4
JP	505	1	2	4
JP	128	1	3	4
JP	202, 213, 223, 241, 249, 767, 786, 825, 826, 846, 871, 883	1	4	4

A set of 337 segregating lines for powdery mildew resistance was scored individually in F_6, F_7 and F_8 generations. Of these, 54 recombinations with high resistance and 38 with resistance against powdery mildew were isolated in F_8 generations.

The remaining 245 lines reverted back to susceptibility gradually. Schroeder and Provvidenti, have also observed that complete immunity against powdery mildew conferred by er er genes was broken down by as yet unidentified reasons[3]. This breakage of resistance is still to be postulated.

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In vivo influence of culture filtrate toxin of *Fusarium solani* (MART.) Appel & Wollenw. *emend.* Snyd. & Hans. on the disease syndrome of tomato seeds and seedlings

(Key words : *Fusarium solani*/culture filtrate)

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Abstract

Culture filtrates of two isolates of *Fusarium solani* were tested to assess mortality of seeds and seedlings of two tomato cultivars—Marglobe and Pusa Ruby. The culture filtrate affected an increase in the incidence of pre-and post-emergence killing as well as wilting of tomato seedlings. Culture filtrates of several isolates of *F. solani* obtained from rhizosphere and non-rhizosphere soils of tomato were found to be equally toxic causing wilting of 6-week old tomato cuttings. Influence of toxin production and its reaction on host varied with age of the culture, the isolate and the variety of the host taken.

Introduction

Root rot disease of tomato due to *Fusarium solani* is an economicaly important disease as the pathogen present in the soil causes wilting of mature plants[1, 2]. The intricate host-parasite relationship has not been worked out comprehensively till now. Wall degrading enzymes do act as an agent of cell breakup. However, the ability of the fungus to secrete such enzymes does not always correspond with the intensity of disease incidence[3]. Therefore, it is proposed to inquire into the role of toxic metabolites present in the culture filtrate of *Fusarium solani* on the expression of disease syndrome in tomato seedlings. In this communication the results of studies on the influence of culture filtrate metabolites of nine isolates of *Fusarium solani* on pre-and post-emergence killing and wilting of two cultivars of tomato are reported.

Material and Methods

Two isolates of *Fusarium solani* designated as 1 (I.M.I. No. 267376) and 2 (I.M.I. No. 267377) were obtained from Commonwealth Mycological Institute, Kew Surrey, U.K. and seven isolates designated as 3, 4, 5, 6, 7, 8 & 9 isolated from rhizosphere and non-rhizosphere soils of tomato fields were taken. As they exhibit different parasitic vigour against two cultivars of tomato (Marglobe and Pusa Ruby), they were included in this study. Isolates 1 and 2 were grown on liquid Czapek's sucrose nitrate medium for 20 days at 28 ± 2°C. After an interval of 5, 10, 15 and 20 days each, which were autoclaved at 20 p.s.i. for 20 min. before their bioassay was made on seeds and seedlings to find out the effect of culture filtrate toxin on 6-week old seedlings of tomato (Table 1).

Seedling of both the cultivars of tomato were kept in glass tubes containing 5, 10, 15 and 20 day old autoclaved culture filtrates. Five cuttings of seedlings with 3–4 expanded leaves

were immersed in 8 ml portions of filtrate kept in sterile 10 ml glass tubes. In case of control, seedlings were kept in sterilized distilled water. Hourly observations were made upto 24 h on an average of 5 replications by noting down the symptoms, using the following arbitrary scale; 0 = no symptoms; 1 = wilting of all leaves except terminal bud; 4 = complete wilting of all leaves without dessication of leaves; 5 = complete wilting and death of seedlings.

Effect of crude toxin in 20 day old autoclaved culture filtrate of seven more isolates numbered 3 to 9 of *F. solani*, isolated from rhizosphere and non-rhizosphere soils of tomato fields, was found out as per methods outlined above on seedling mortality by noting down their wilting index (Table 2).

Twenty-day old autoclaved culture filtrates of isolate 1 ad 2 were examined for pre-and post-emergence killing of Marglobe and Pusa Ruby varieties of tomato seeds by placing 25 seeds of each variety in separate petriplate containing filtrate-soaked filter paper. For each treatment, 4 replicates were taken (Table 3). Control seeds were kept on filter paper wetted with tap water.

Results and Discussion

The results as presented in Table 1 show that in general, culture filtrate crude toxin of both the isolates was severely toxic to both the cultivars of tomato and the toxicity varied with age of culture.

Table 1–Effect of 5, 10, 15, 20 day-old culture filtrates of two isolates 1 and 2 of *Fusarium solani* on wilting of Marglobe & Pusa Ruby cultivars of tomato cuttings.
(Observations after 24 h)

Isolates	5-day old culture filtrate		10-day old culture filtrate		15-day old culture filtrate		20-day old culture filtrate	
Cultivars	M.	P.R.	M.	P.R.	M.	P.R.	M.	P.R.
1.	0	0	3	1	3	2	4	4
2.	0	0	3	2	4	2	5	4
Control	0	0	0	0	0	0	0	0

Table 2–Effect of 20 day old culture filtrate of 7 isolates (3–9) of *Fusarium solani* on tomato seedling mortality

Isolates	Observations after 12 h	Observations after 24 h
3	5	5
4	3	4
5	5	5
6	4	5
7	5	5
8	4	5
9	2	3
Control	0	0

Table 3–Effect of 20 day old cultrue filtrate of two isolates of *F. solani* on pre-and post-emergence mortality of Marglobe and Pusa Ruby cultivars of tomato

Isolate	MARGLOBE			PUSA RUBY		
	% of pre-emergence killing	% of post emergence killing	% of total killing	% of pre emergence killing	% of post-emergence killing	% of total killing
1	54	43	97	59	30	89
2	56	44	100	63	35	98
Control	16	14	30	30	10	40

However, isolate 2 was more toxic than isolate 1. Cultivar Marglobe was more affected than Pusa Ruby by both the isolates as the wilting symptom started in Marglobe cutting in just 5 h and wilting of all leaves took place except for the terminal portion. Later on, the rotting of collar region also took place when continuously kept for 24 h resulting in complete wilting and damping off with the collapse of plant. Twenty-day old culture filtrate had the maximum toxicity against both the cultivars. Since the autoclaving of the culture filtrate did not affect its toxicity adversely, it is confirmed that the crude toxic is thermostable in nature.

Results as summarized in Table 2 show that the culture filtrate crude toxins of the isolates 3, 5, 6, 7 and 8 *Fusarium solani* are more toxic than the isolates 4 and 9.

Filtrates of isolates 1 and 2 adversely affected germination of seeds of both the varieties of tomato. The pre-and post-emergence killing was to the tune of 89-100%. However, the pre-emergent death of seeds was more pronounced in both the cultivars. Filtrate of isolate 2 was more toxic than that of isolate 1. Varietal difference show that Marglobe is more susceptible than Pusa Ruby. From the above experiments it is also concluded that the culture filtrate primarily had more toxic effect on the seeds than on the seedlings.

The above results are in conformity with the results obtained by Joffe[4, 5] and Kumar and Mahmood[6] for the culture filtrates of *Fusarium poae, F. sporotrichoides* on pea, wheat, bean, barley seeds and *Collectotrichum dematium* on seeds, seedlings and fruits of chilli, respectively.

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Nature of interaction between *Rhizobium* and *Glomus caledonicum* in chickpea (*Cicer arietinum* L.)

(Key words : *Glomus caledonicum/Rhizobium*//chickpea/synergistic association)

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Abstract

Glomus caledonicum and *Rhizobium* sp. when inoculated individually or in combination enhanced plant growth, phosphorus uptake from different sources, nitrogen fixation and N. P and Zn content of the chickpea (*Cicer arietinum* L.) plants. With respect to most of the parameters, combined effect was more than the sum total of the individual effect indicating the synergistic association between the symbionts. In combined inoculation, *Rhizobium* and *G. caledonicum* enahaned the root infection by each other.

Introduction

Both vesicular-arbuscular mycorrhizal (VAM) fungi and *Rhizobium* colonize roots of leguminous plants and establish a symbiotic association[1, 2]. VAM fungus is an obligate biotroph and draws its nutrients only from living cells of the host with no saprophytic activity in soil[3], whereas rhizobial association is biotrophic only to the extent of its association with host. *Rhizobium* sp. forms the nodules on the host root and draws its nutrients form living cells of the host but they can also grow in soil as saprophytes particularly under the influence of host root exudates[4].

VAM fungus is known to improve the nutrient uptake such as P, Zn, and Cu by reducing the distance so that nutrients can diffuse to plant roots[5, 6]. However, *Rhizobium* bacterium after forming the nodules in roots fixes the atmospheric nitrogen in the roots[7]. But for the effective growth of this bacteria, phosphorus is the major nutrient[8] and it can be made available either through phosphatic fertilizers or by biological agents like mycorrhizae particularly in P deficient soil[9]. Asai (1944) suggested that mycorrhizae were necessary precondition for effective nodulation in legumes and the same has also been supported by many workers[11, 12]. The present study deals with the nature of interaction i.e. antagonistic, additive or synergistic between *Glomus caledonicum* and *Rhizobium* sp. in association with the chickpea host particularly with respect to their individual and combined effect on host growth, nutritional status, nitrogenase activity, P availability, its uptake and translocation.

Materials and Methods

Glomus caledonicum Herd. and Trappe, maintained on maize grown in sterile sand with Hoagland's solution with half strength of phosphorus for a minimum period of 90 days was

used as a VAM inoculum. It was inoculated with chickpea (*Cicer arietinum* L. cvs. Pant G-114 and BG 209) @ 200 spores/plant by placing the inoculum as padding beneath the seed[13] at the time of sowing. Spore washings of *G. caledonicum* mixed with the soil served as control. *Rhizobium* strain IC-76, obtained from International Crop Research Institute for Semi-Arid Tropics, Hyderabad, India, was applied as a seed treatment @ about 10^3 cells/seed.

Initially six seeds were sown in each pot (2 kg cap.) having P-deficient loam soil (pH-6.8, p–3.5 ppm, N-0.143%, Zn – 0.70 ppm) with individual and combined inoculum of both the organisms at 25-27°C in a glass house. After 15 days of sowing only four plants were allowed to grow. All the treatments were replicated five times.

Plant growth responses (length, fresh and dry weight of root and shoot, nodulation) and nutrient (P, N and Zn) contents were studied 30 and 60 days after sowing. However, yield was recorded at the majurity of crop i.e. 105 days after sowing. Mycorrhizal infection was assessed after staining the roots[14]as per the method of Biermann and Linderman[15]. Phosphorus, nitrogen and zinc were estimated in plants as per the method of Murphy and Riley[16], Jackson[17] and atomic absorption spectrophotometry respectively. Nitrogenase activity in root was assessed by acetylene reduction method[18].

^{32}p-labelled phosphoric acid (after adjusting the pH to 6.8 by 0.01 NaOH) and ^{32}p-phosphatic fertilizers (monocalcium and dicalcium phosphate) were applied @ 200 μCi/pot and 1.47mCi/pot (@ 40 kg/ha after 53 and 38 days of sowing respectively. ^{32}p was estimated after one week of application in both the cases through Gas Proportional Counter after digesting the plant tissues in triacid (HNO_3 : H_2SO_4 : $HC10_4$ (60%), 10 : 1 : 4). Rock phosphate (non-radio active) was used as insoluble source of phosphorus and applied in the soil @ 40 kg/ha before sowing. The P content was estimated after 45 days of plant growth[16].

Results and Discussion

As shown in Tables 1–3, plant vigour and P uptake by the plants improved significantly with the inoculation of both the symbionts. When compared individually, effect of *G. caledonicum* was more pronounced with respect to all parameters except nodulation, N content in root and shoot and nitrogenase activity. Inoculation of one symbiont improved the colonization by the other. When inoculated together, combined effect of symbionts on plant vigour, nutritional status of the plant, P-uptake and nitrogen fixation was usually more than the sum total of their individual effects. It indicates towards synergistic interaction between VAM fungus and *Rhizobium* . In some parameters (Table 1 and 2), the interaction looked additive where combined effect of symbionts was less than the sum total of individual effects. However, keeping in view that such variations are always expected in biological interactions, overall interaction of VAM and *Rhizobium* in chickpea can be considered as synergistic. Synergistic effect of *Rhizobium* and VAM may be due to the fact that these symbionts improved overall health and vigour of the plant through enhancement of the nutritional status[12, 14, 20] and in turn make the plant more prone towards further colonization of roots by these symbionts as their relationship with the plant is biotrophic. In general, healthy plants with a rich nutritional status showed greater colonization by the biotrophs[12].

Table 1- Nature of interaction between VAM and *Rhizobium* in chickpea with respect to different growth parameters, mycorrhizal infection, and yield.

Parameter	Per cent increase over check *			Nature of effect
	Rihizobium alone	VAM alone	VAM + *Rhizobium*	
Natural soil				
Shoot height	18	47	54	Additive
Fresh Shoot wt.	51	79	126	Additive
Dry Shoot wt.	12	44	84	Synergistic
Root length	15	45	69	Synergistic
Fresh root wt.	14	70	110	Synergistic
Mycorrhizal inf.	25.1	90.5	149.7	
Yield	27.8	86.6	135.0	Synergistic
Sterilized soil				
Shoot height	4	10	14	Synergistic
Fresh shoot wt.	26	41	70	Synergistic
Dry shoot wt.	15	35	42	Additive
Root length	30	62	90	Additive
Fresh root wt.	7	39	57	Synergistic
Dry root wt.	17	52	65	Additive
*Mycorrhizal inf.	0	2870	3400	Synergistic
Yield	20	49.2	101.5	

* Based on the observations recorded on two varieties of chickpea at two growth stages i.e. 30 and 60 days after sowing except yield which was recorded at maturity.

** Because of no mycorrhizal infection in chek plants and only *Rhizobium* inoculated plants in sterilized soil, the per cent values were calculated after assuming 1% infection in these treatments.

Table 2-Nature of interaction betweenVAM and *Rhizobium* in chickpea varieties with respect to nodulation, nitrogenase activity, P, Zn and N content.

Parameter	Per cent increase over check *			Nature of Effect
	Rhizobium alone	VAM alone	VAM + *Rhizobium*	
Nodulation	62	40	90	Additive
Nitrogenase activity	114	47	170	Synergistic
Phosphorus content				
Shoot	35	51	131	Synergistic
Root	35	75	98	Additive
Zinc content				
Shoot	13	35	57	Synergistic
Root	1	30	57	Synergistic
Ntrogen content				
Shoot	16	11	26	Additive
Root	12	6	29	Synergistic

Based on the observations recorded on two varieties of chickpea.

Table 3- Nature of interaction between VAM & *Rhizobium* in chickpea var. Pant.G. 114 and BG-209 with respect to phosphorus uptake.

Parameter	Per cent increase over check *			Nature of effect
	Rhizobium alone	VAM alone	VAM + *Rhizobium*	
Var. Pant G-114				
P content				
i) ^{32}p phosphoric * acid	31.1	263.0	1019.0	Synergistic
ii) ^{32}p Monocalcium** phosphate	13.8	75.6	149.0	Synergistic
iii) ^{32}p Dicalcium** phosphate	9.8	35.0	72.3	Synergistic
iv) Rock phosphate**	3.3	20.2	34.8	Synergistic
Var. BG-209				
P content				
I) ^{32}p phosphoric* acid	57.7	229.5	846.7	Synergistic
ii) ^{32}p Monocalcium phosphate**	28.9	89.2	138.0	Synergistic
iii) ^{32}p Dicalcium phosphate **	16.0	45.1	71.7	Synergistic
iv) Rock phosphate**	3.8	23.0	37.2	Synergistic

* After 60 days of sowing.
** After 45 days of sowing

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Effect of salmon calcitonin on serum calcium and inorganic phosphate levels of male *Anabas testudineus* Bloch

(Key words : salmon calcitonin/hypocalcemia/*Anabas testudineus*)

Abstract

Serum calcium and inorganic phosphate levels were estimated after 15, 30, 60, 90 and 120 minutes of salmon calcitonin (1 MRC unit) administration in male *Anabas testudineus*. Ca and iP recorded a transient minor decrease ($P < 0.05$), only after 60 minutes of calcitonin administration.

The ultimorbranchial gland (UBG) of fishes have been shown to possess potent hypocalcemic activity in rat bioassay[1–5] and calcitonin (CT) has been extracted in large quantities from salmon[6] and eel[7] UBG. Conflicting reports regarding the role of CT in calcium regulation of teleosts exist and the problem remains unsettled. Certain workers have shown that fish/mammalian CT reduces blood calcium level in fish[8–15] while others have demonstrated that it fails to elicit hypocalcemia in fish[14–19]. Yet another group of workers believe that CT is not involved in hormonal regulation of calcium in fishes rather it is related to sexual maturation[20–21] and in hydromineral balance[4].

UBG extract of *Anabas* induces hypocalcemia when tested in rats. In *Anabas* this gland displays a seasonal change and becomes hyperactive during breeding period and evidently secretes more CT to combat hypercalcemia caused under the influence of estrogen[22]. These observations prompted us to examine the effect of exogenous salmon CT on the serum calcium and inorganic phosphate levels of *A. testudineus*.

Fifty male *Anabas* (body weight 60 ± 5 g) were sorted out and maintained in tap water in glass aquaria at temperature of 20 ± 2°C under a day light period of 10.31 to 10.34 hours for a weak prior to use. Then the fishes were divided into two equal groups.

Group A : Received 0.5 ml of vehicle (0.6% NaCl and 1% gelatin in distilled water).

Group B : Received 1 MRC unit of salmon calcitonin (Armour Pharmaceutical Company USA, lot No. K713-881) dissolved in 0.6% NaCl and 1% gelatin in distilled water.

The fishes in groups of 5 were killed at 15,30,60,90 and 120 minutes after receiving the injection and the blood samples were taken by sectioning of caudal peduncle under anaesthesia with tricaine (MS-222, Sandoz, Basel). Sera were separated by centrifugation at 3500 rmp. Serum calcium and inorganic phosphate were analysed according to Trinder's[23] and Fiske and Subbarow's[24] methods respectively.

Differences in the serum calcium and inorganic phosphate levels between vehicle and CT injected specimens were evaluated using Student's t-test.

A single injection of 1 MRC unit of salmon CT to male *Anabas testudineus* results in a minor decrease ($P<0.05$) in serum calcium and inroganic phosphate level after 60 minutes. At other time intervals these values record no change (Table 1; Fig. 1). Serum calcium and inorganic phosphate levels remained unchanged among fishes which were injected vehicle.

The hypocalcemic effect of CT in fishes remains unsettled and the results are equivocal. Pang and Picford[14], (in *Fundulus heteroclitus*), Pang[15] (in *Anguilla rostrata*), Yamauchi *et al.*[16] (in *Anguilla japonica*), Wendelaar Bonga[17] (In *Gasterosteus aculeatus*), Hirano *et al.*[18] (in *Anguilla japonica*) and Bjornsson and Deftos[19] (in *Gadus morhua*) have reported that administration of CT does not induce hypocalcemia. On the contrary, Chann *et al.* (in *Anguilla anguilla*), Peignoux–Deville *et al.*[10] (in *Anguilla anguilla*), Mathur, (in *Channa punctatus*), Wales and Barrett[12] (in *Carassius auratus* and *Anguilla anguilla*) and Wales[13] (in *Anguilla anguilla*) have observed CT induced hypocalcemia. Further, Wendelaar Bonga[25] working on freshwater stickleback adapted to low calcium environment, reported a transient decrease in free calcium fraction of blood plasma, though there was no decrease in total calcium.

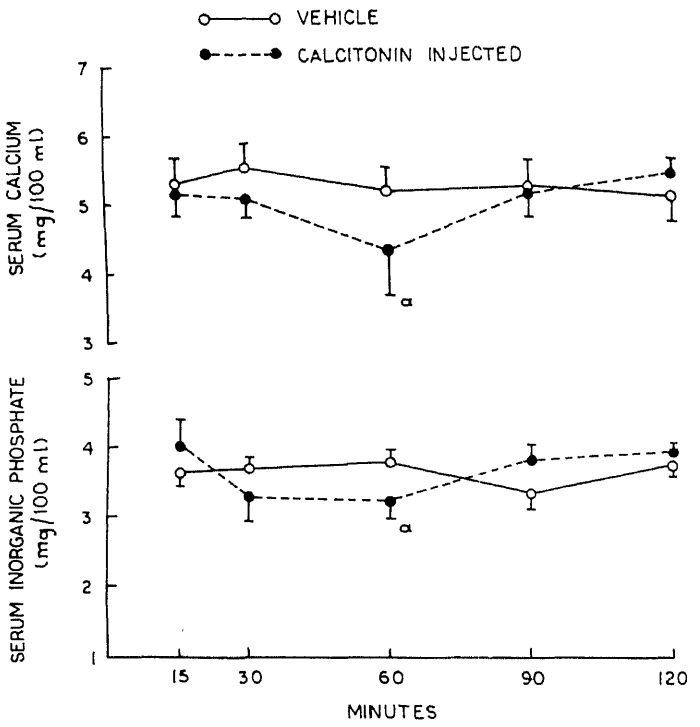

Fig. 1 Serum calcium and inorganic phosphate levels of male *Anabas testudineus*, after a single injection of salmon calcitonin. Each point indicates mean ± S.D. of five determinations; a indicates significant response, $P < 0.05$.

Table 1–Effects of a single injection of salmon calcitonin on serum calcium and inorganic phosphate levels of male *Anabas testudineus*.

Time	Serum calcium (mg/100 ml)		Inorganic phosphate (mg/100 ml)	
	Group A (control)	Group B (calcitonin)	Group A (control)	Group B (calcitonin)
15	5.320 ± 0.395	5.106 ± 0.316	3.674 ± 0.155	4.049 ± 0.332
30	5.592 ± 0.368	5.150 ± 0.316	3.694 ± 0.163	3.314 ± 0.345
60	5.218 ± 0.297	4.374 ± 0.644[a]	3.802 ± 0.183	3.258 ± 0.245[a]
90	5.324 ± 0.371	5.224 ± 0.318	3.422 ± 0.221	3.856 ± 0.225
120	5.150 ± 0.130	5.558 ± 0.370	3.780 ± 0.068	3.978 ± 0.166

Each value represents mean ± SD of 5 specimens; a, indicate significant response compared to controls ($P<0.05$)

Fenwick and Lam[26] ascribed an antihypercalcemic rather than hypocalcemic role to CT in *Periophthalmodon schlosseri*, to correct excessive calcium levels. Bjornsson and Deftos[19] failed to observe such a role of calcitonin in cods. According to them CT injections did not delay the onset of hypercalcemia during environmental calcium challenge nor did such injections have any antihypercalcemic effect on already hypercalcemic fish and the endogenous plasma level of CT remained unaffected during hypercalcemia. This prompted them to suggest a physiological function of CT not related to blood calcium.

In the present study on the freshwater perch *Anabas testudineus*, CT induces minor hypocalcemia and hypophosphatemia after 60 minutes since the initiation of the experiment which is transient and not very significant. Mathur[11] and Wales and Barret[12] have reported hypocalcemia 1 hour after intraperitoneal injection of porcine and salmon CT in *Channa punctatus* and gold fish respectively. In grey mullets Fouchereau-Peron *et al.*[27] have observed hypercalcemia in response to low dose (0.1 ng) and hypocalcemia against high dose (0.5μg) of salmon CT. To this unusual finding they have ascribed the reason that CT in teleosts is principally a hypercalcemic hormone and may be important in processes other than calcium regulation.

Certain workers[19–21] have ascribed a specific role of CT in sexual maturation and ovulation of females in a number of teleosts without any apparent relationship with plasma calcium level.

In *Anabas testudineus*[22] the serum calcium and inorganic phosphate levels increase remarkably in both sexes with gonadal maturation (increase in gonosomatic index) which record an abrupt fall with spawning. These values are always high in females than males. With increased level of calcium and phospate the ultimobranchial glands of *Anabas* exhibit hyperactivity which leads to regression during postspawning phase. These findings in *Anabas* suggest a calcium-dependent sex-related role of ultimobranchial gland and calcitonin.

Thus, keeping in view the above facts, it is suggested that more data are needed to settle the role of calcitonin (if any) in calcium homeostasis or/and in reproduction in fishes.

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Occurrence of Tarsonemid mite as a pest of potato in Farrukhabad (U.P.)

(Key words : potato pest/*Polyphagotarsonemus latus* /Kharif season/incidence)

Abstract

Systematic field surveys show that tarsonemid mite has attained a status of a major pest of potato in the Indo-Gangetic plains of India in Farrukhabad district (U.P.) where intensive cultivation of potato and collateral host plants of this mite is practised. *Solanum melongena, Capsicum annum, Tagetes* sp. and *Solanum nigrum* were recorded as collateral hosts of this mite.

Tarsonemid mite, *Polyphagotarsonemus latus* Bank also known as broad mite, yellow tea mite, chilli muranai mite and tropical mite, is a serious pest of kharif potato in peninsular India. Its damage popularly known as 'Tambra' disease of potato in Maharashtra and Karnataka is responsible for 100% damage to foliage and 60% reduction in the yield during kharif season[1,2]. However, no information is available about this mite on potato in Indo-Gangetic plains of India.

Systematic field surveys were conducted in 12 villages of Barhpur, Kaimganj and Kamalganj blocks in Farrukhabad district during 3 crop seasons from 1984 to 1987. Two surveys were conducted in each crop season during 4th week of December and 3rd week of February. Observations were recorded on four varieties popularly grown in the region, *viz.*, K. bahar, K. Chandramukhi, K. Badshah and G-4. In each field 100 random selected plants were critically examined for the damage by this mite. Besides, leaf samples were collected in polythene bags for population count under microscope

The incidence of this mite on potato crop varied during different years. During December 1984 and 1985 when winter rains were plenty and the crop was given heavy spraying for protection against late blight, the incidence was confined only to a few fields of Nanglakhairbund, Barhpur, Nawadia villages and Farrukhabad town. The damage was confined to 4-5 top leaves. The infested leaves were reduced in size and became leathery and their margins turned downwards. The mite population on infested leaves varied from 6 to 25/leaflet. In certain fields 1 to 5% plants were infested (Table 1). No infestation of this mite was recorded during February 1985 and 1986 as most of the potato foliage were already damaged by late blight or they were heavily treated with pesticides.

During the 4th week of December, 1986 when winter rains did not occur and most of the crops were not sprayed due to non-incidence of late blight, the occurrence of this mite was very severe in certain localities of Barhpur block. All the four popular varieties in the region were affected by this mite. In severely infested fields all the leaves were affected by this mite and characteristic copper colour deposits were clearly visible on the lower surface of the leaves. In poorly infested fields only top 4 to 5 leaves were affected. The damage was more severe on the 65 to 70 days old crops where 23 to 100% plants were infested. Its infestation on younger plants were either poor or entirely absent (Table 1).

Table 1–Occurrence of *Polyphagotarsonemus latus* Bank on potato crop in Farrukhabad

Block	Village	Potato variety	% crop damaged by mite			
			1984 IV week December	1985 IV week December	1986 IV week December	1987 III week February
Barhpur	Kyiabuth	G-4	0	0	10-100	—
		K. Bahar	0	0	60-100	—
		K. Chandramukhi	0	0	70	—
		K. Badshah	0	0	—	40
	Nanglakhairbund	K. Badshah	0	0	2	0
		K. Bahar	2	1	100	—
	Farrukhabad	K. Bahar	—	1.5	—	—
	Deorampur	K. Bahar	0	0	54	—
		K. Badshah	0	—	1	2
	Barhpur	K. Bahar	0	5	0	0
	Bholepur	K. Badshah	0	0	0	45
	Nawadia	K. Bahar	0	1	23	—
	Bankharia	K. Bahar	1	0	100	—
	Rakha	K. Badshah	0	0	—	50
Kamalganj	Jahanganj	K. Bahar	—	—	11	—
		K. Badshah	—	—	—	45
	Patiunja	K. Bahar	—	—	0	0
Kaimganj	Manjhana	K. Bahar	0	0	0	0

Most of the fields in Jahanganj and Patiunja villages (Kamalganj block) were free from this mite during December, 1986. Only in a few fields the damage was upto 1%. Potato crops in Manjhana village (Kaimganj block) were free from mite infestation.

During the 3rd week of February, 1987 the mite damaged unsprayed crops of K. Badshah to the extent of 40 to 50% in Kiuyabuth, Bholepur, Rakha (Barhpur block) and Jahanganj (Kamalganj block) villages. The infested leaves were only with copper colour deposits on the lower sides. The mite population on such leaves were 30 to 150/leaflet. All stages of the mite were present on the leaves. The eggs were transparent and oval in shape. The young ones were white and adults were yellowish in colour. *Solanum melongena, Capsicum annum, Tagetes* sp. and *Solanum nigrum* were found to be collateral host plants of the mite.

It is concluded from the survey that *Polyphagotarsonemus latus* Bank has attained the status of a potential pest of potato around Farrukhabad and in other areas where intensive cultivation of potato and of collateral host plants of the mite is practised.

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Effect of amino compounds on nitrate reductase, glutamine synthetase and glutamate dehydrogenase activities in moong (*Vigna radiata*) nodules

(Key words : amino acids/ammonia assimilation/nitrate reductase/*Vigna radiata*)

Abstract

Amino compounds (aspartate, glutamate, glutamine, asparagine, tryptophan and ammonium, 1 mM each) were supplied for 17 h to field grown 29 days old intact moong (*Vigna radiata* L. cv. ML-131) plants in the absence and presence of 100 mM sucrose in solution culture; and their effect was studied on *in vivo* nitrate reductase (NR), glutamate dehydrogenase (GDH) and glutamine synthetase (GS) activities in nodules. Glutamine, asparagine and ammonium were more effective in lowering NR activity. Asparagine drastically decreased whereas glutamate increased GDH activity. All the amino compunds, except glutamate, reduced GS activity. Sucrose addition reduced NR activity and increased GS and GDH activities, irrespective of the presence of amino compounds, except tryptophan which, in the presence of sucrose, decreased GDH activity considerably.

Extensive data is available pertaining to the effect of ammonium (NH_4+) and amino acids on nitrate utilization by roots and shoots of higher plants[1–3]. The inhibitory effect of NH_4^+ on nitrate utilization is considered to be due to high energy requirements of its incorporation[4]. The exogenous addition of sugars to incubation medium has been found to increase N_2 ase activity in nodules of moong, lentils and soybeans[5–7]. A review of literature reveals that the effect of exogenously supplied amino acids on nitrate and ammonia assimilation in legume nodules remain yet to be established. The present study, therefore, deals with the effect of exogenous addition of NH_4^+ and amino acids on nitrate reductase (NR), glutamine synthetase (GS) and glutamate dehydrogenase (GDH) activities in moong nodules in the presence and absence of sucrose.

Amino compounds aspartate (Asp), glutamate (Glu), glutamine (Gln), asparagine (Asn) tryptophan (Trp) and NH_4^+ (as NH_4Cl), 1 mM each, were supplied to 29 day old field grown intact moong (*Vigna radiata* L. cv. ML-131) plants in solution culture for 17 h at 30°C in the presence or absence of 100 mM sucrose. The nodules were then detached from the roots, washed with distilled water, blotted dry and activities of NR, GDH and GS were assayed. *In vivo* NR activity was assayed by the method of Jaworski[8]. For assay of GDS and GS, the nodules were macerated in 5 ml of phosphate buffer (pH 7.5) containing 5 mM cysteine. This was followed by centrifugation at 10,000 g for 10 min at 0-4°C. Th extract was used to determine the activities of GDH and GS by methods of Bulen[9], and Manamori and Matsumoto[10] respectively.

Asparagine (Asn), glutamine (Gln), and NH_4^+ decreased whereas glutamate (Glu), increased the activities of NR, GDH and GS (Table 1). Tryptophan (Trp) had no effect on NR and GDH activities but decreased GS activity. The addition of sucrose alone reduced NR activity but enhanced GDH and GS activities in the control. Wong[6] observed that sucrose lowered NO_3^- accumulation by the lentil roots, thereby, lowering the *in vivo* NR activity. The inhibition of

Table 1–Effect of amino compounds (1 mM) in the absence or presence of sucrose (100 mM) on NR, GDH and GS activities in moong nodules.

Amino Compound	NR	GDH	GS
	(μ moles of NO_2 formed/ h/g nodules fresh wt)	(μ m moles of NADH oxidised/ min/g nodules fresh wt.)	(μ m moles of –glutamyl hydroxynate formed/min/g nodules fresh wt.)
Control	0.42 (0.29)	7.00 (9.83)	0.80 (0.93)
Asp	0.36 (0.30)	5.99 (9.27)	0.60 (1.20)
Glu	0.58 (0.39)	8.67 (7.40)	0.86 (1.13)
Gln	0.20 (0.09)	5.40 (7.40)	0.60 (0.83)
Asn	0.18 (0.10)	3.88 (6.79)	0.43 (1.16)
Trp	0.42 (0.10)	6.96 (1.90)	0.60 (0.93)
NH_4^+	0.26 (0.24)	5.05 (5.68)	0.60 (0.66)

Different compounds (1 mM) in the absence or presence of sucrose (100 mM) were supplied to 29 days old field grown moong plants with intact nodules at 30°C. After 17 h, the nodules were detached, washed, dried and the activities of NR, GDH and GS were determined. The figures in the parentheses indicate the results in the presence of 100 mM sucrose.

NR activity by sucrose in the present results might be due to lowered accumulation of NO_3^- in the nodules. In dwarf bean roots, NR activity got stimulated with Asp and Trp, decreased with Asn and Gln, whereas Glu had no effect[11]. The present results showed that the decrease in NR activity by amino acids was higher in the presence of sucrose. However, compared to control (minus sucrose), NR activity remained unaffected in the presence of Glu + sucrose, thus suggesting that Glu addition antagonised the effect of sucrose. The inhibition of NR activity due to NH_4^+ remained unaffected by sucrose addition.

Asp, Gln, Asn, and NH_4^+ decreased GS and GDH activities and the maximum decrease was observed with Asn. It is well established that in the presence of N source, nitrogenase shows depressing effect. Thus the possibility, that reduction in the activity of GDH and GS enzymes may be due to non-availability of ammonia through nitrogen fixation, cannot be ruled out. It is also evident that sucrose addition relieved the depressing effect caused by Asp, Gln and Asn. However, sucrose in the presence of tryptophan, lowered GDH activity considerably. It is well known that sugars are donors of carbon skeletons of amino acids[12].

Thus, it appears from the present results that the increase in GDH and GS activities in the presence of sucrose, might be due to increased availability of carbohydrates.

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